CHOLERA INFANTUM,

ITS

CAUSES AND TREATMENT.

BY

DAVID KING, JR. M.D.

Duo in Morbis præstanda sunt; adjuvare, aut saltem non nocere.—HIPP. EPIDEM. TRANS.

DISSERTATION.

" What are the causes and nature of CHOLERA INFANTUM, *and the best mode of treatment to be employed therein ?"*

CHOLERA INFANTUM has not hitherto received that attention which its prevalence and fatality demand. Its literary history includes only the recorded experience of a few medical observers, whose researches tend more to illustrate the symptoms and progress of the disease, than to unfold its pathological states and relations. Its attacks are almost entirely confined to teething children, especially during the period which intervenes between their fifth and twentieth month. It prevails, during the warm season, in the large towns of Europe and the United States, and in climates within the tropics. In the United States, its prevalence is mostly confined to large and crowded cities, between the months of May and October. In the country it seldom makes its appearance, except occasionally in villages where the houses are very compact, or in localities rendered unhealthy by their moisture and low situation. It commences in the southern States, the Carolinas, Alabama, Louisiana and Mississippi, in April or May; in Pennsylvania, Maryland, Virginia, Kentucky and Ohio, in June; in New England, in August, where the disease is most frequent in September.

Its fatality is very marked; it being about one fourth of all the deaths among children in our large cities. Its ravages seem to increase, as we approach towards the equator. In the city of Philadelphia, the average number of deaths, among children under two years of age, is two hundred annually. In 1823, two hundred and fifty children died of cholera infantum in Baltimore. In Cincinnati, its fatality is very great. Dr. Cartwright remarks, that the traveller, on visiting the burial places of that city, is astonished at the immense number of children's graves.

Cholera infantum most commonly commences with vomitings and purgings, which are associated with high febrile excitement. Diarrhœa, in some instances, precedes, for several days, the occurrence of vomiting, but most frequently they occur simultaneously, or alternate with each

other. Functional disturbance of the nervous system, and slight irritation of the mucous membrane of the alimentary canal, usually precede the vomitings and purgings. The matters vomited consist, at first, of a greenish or yellow fluid, and afterwards of a frothy mucus, and of drinks that may be given to the patient. The alvine discharges occur from three to twenty times in the course of twenty-four hours. The dejections vary in appearance, sometimes consisting of an abundant secretion of mucus, slightly tinged with bile, and mixed with pieces of curd, if the child have been fed on milk; sometimes they consist of a copious exhalation of serum, containing small portions of mucus; sometimes they are slimy and bloody, without any tinge of bile. The fæcal matter is mostly retained in the intestines; small portions of it occasionally escape with the other discharges. The discharges want the true fæcal smell; they are, mostly, sour or putrid, like water in which putrid meat has been washed. As the disease advances, the secretion of bile is suspended.

This irritability of the alimentary canal is accompanied by a remittent fever, whose exacerbations are highest in the evening. The patient is restless and irritable. The pulse is small, quick, and frequent, sometimes corded. The thirst is intense, in all stages of the disease. The tongue is covered at first with a thin white fur, but gradually acquires a dry, red, and polished appearance, particularly when the disease is prolonged.

The patient evidently experiences the same indescribable distress about the epigastric and præcordial regions, which is felt in the cholera of adults. In severe cases, spasms occur in the abdominal muscles, and in the muscles of the extremities. The patient draws up his feet, and is uneasy in every position.

The brain is, early, sympathetically affected, as indicated by a tendency to stupor or delirium. The eyes are either fierce, or dull and inanimate, and the patient sleeps with them half open. The head and abdomen are hot, while the extremities are cold.

In the most violent form of the disease, the vital powers are suddenly exhausted, the extremities become cold and damp, the surface of the body collapsed, and death ensues within a single day. Fatal exhaustion rarely occurs, however, before the fifth or sixth day.

In many cases, the vomiting and severe symptoms gradually diminish, a general moisture of the skin and an equal distribution of temperature ensue, and convalescence begins in five or six days from the commencement. If convalescence do not occur in a few days, rapid emaciation takes place. The whole adipose substance appears to be absorbed. The skin is dry and harsh; on the forehead it appears tight, as if bound to

the bone ; on the inner part of the thighs, and over the abdomen, it has a wilted appearance. The countenance is contracted, and of a deadly paleness. The nose is sharp, and the lips thin and shrivelled, as in old age. The extremities are cold and damp, and the head and abdomen preternaturally hot. The thirst is excessive, and the desire of cold drinks constant. The irritability of the stomach is so great, that cold water, the only drink which the patient does not refuse, is retained but a few moments after being taken. The disease may continue thus for many weeks, and yet recovery may occur from such excessive exhaustion and emaciation.

Frequently, a fatal termination is produced by the affection of the brain, which assumes the form of acute hydrocephalus. The little patient rolls his head about when awake ; when asleep, the eyelids are half closed and the eyes turned up. He gradually sinks into a state of insensibility, so that, as noticed by Dr. Rush, flies alight on the eyes when open, without exciting a movement of the eyelids for their removal. Death generally occurs in a paroxysm of convulsions.

Another termination results in the more protracted forms of the complaint. The disease seems to fix with a firmer grasp upon the intestines. The alvine discharges are dark and offensive, and so acrid as to excoriate the parts about the anus. The function of digestion is suspended, and the ingesta pass through the intestines in an unaltered state. The thirst is excessive. Aphthæ appear on the tongue and inside of the cheeks, and purple spots on various parts of the skin. The face and feet become œdematous ; the abdomen tympanitic ; the patient dies in a comatose state.

Prognosis.—The prognosis is very uncertain in this disease. A favorable issue may be expected when the liver resumes its functions, and the alvine discharges assume a natural appearance. The renewed secretion of bile, causing dark bilious discharges ; a uniform moisture of the skin, and a natural temperature equally diffused over the surface of the body ; the cessation of the irritability of the stomach and bowels, of the fever, and cerebral disturbance, are among the favorable prognostics. An increase of the cerebral symptoms, of the restlessness and spasms ; convulsions, extreme nervous sensibility, or coma ; a small thready pulse ; hurried respiration ; constant vomiting ; watery, greasy, reddish, and dark flocculose discharges ; stools of pink-colored margin ; cold clammy surface, and haggard countenance, are among the principal unfavorable signs. Dr. Dewees notices, as fatal signs—the appearance upon the chest of a

crystalline eruption, consisting of an immense number of watery vesicles; live worms crawling from the throat, and the thrusting of the hand or fingers into the back part of the mouth, as if to remove something from the throat. Dr. Rush says, " An emaciation of the body to such a degree ·as that the bones come through the skin, livid spots, a singultus, convulsions, a strongly-marked Hippocratic countenance, and a sore mouth," generally precede the fatal termination of this disorder.

Diagnosis.—The disease can rarely be mistaken for other intestinal diseases of infancy. Dr. Jackson says that the disease has been sometimes confounded with an affection of children previous to the age of dentition; arising from some error in diet, or from general debility and indigestion, in the nurse; the alvine discharges being frequent, and consisting partly of fæcal matter, and partly of mucus colored with green bile, of curdled milk, and a watery fluid. This disease is readily distinguished from cholera infantum, by the want of that constitutional disturbance which interrupts the growth and vigor of the body.

Causes of Cholera Infantum.—In the first place, this disease has a specific miasmatic cause. Most endemic maladies, probably, arise from some emanation from the soil, owing to the dissolution of animal and vegetable matter. We know not the nature of these miasms, because they are beyond the reach of our senses and the analyzing processes of art. It is probable, however, that, at first, the animal and vegetable matter is decomposed into atoms of effluvia; and that these atoms of effluvia enter, afterwards, into those peculiar combinations which constitute specific miasms. Our knowledge of the origin of febrile miasm consists, chiefly, in the established fact, that for its production are required a combination of four elements—animal or vegetable matter, atmospherical air, a high temperature, and water in a moderate quantity. But the circumstances of temperature and moisture, elevation, texture and depth of soil, which determine the specific form of the febrile miasm, are beyond the reach of our observation and experiment. We do not know all the combined causes required to produce "hepatitis on the coast of Coromandel, elephantiasis in Malabar, beriberi in Ceylon, Barbadoes leg in the Antilles, goitre among the Alps, the plica in Poland, cretinism in the Vallais," or cholera infantum in the large cities of the United States. The existence of the febrile miasm, producing cholera infantum, is known by its effects. It is confined to particular localities, supplied with materials for the production of miasm. Were the disease attributable to com-

mon causes, as heat, moisture, and atmospherical vicissitudes, this pestis infantum would be a pervading disease, through the whole range of the United States. But its great source is to be found, only, in our large cities, where heat, moisture, a semi-stagnant atmosphere, and filth, or animal and vegetable remains, spread over a large surface, readily produce the *malarious emanation.*

Dr. Eberle has offered two objections to the malarious origin of cholera infantum. 1st. Its occurrence is almost exclusively confined to the period during which the process of primary dentition is going on. 2d. In the eastern cities of America, particularly in Philadelphia, it often prevails extensively during the months of June and July, some time before the ordinary miasmatic diseases are wont to make their appearance in our climate. To the first objection, we answer, that it is not unphilosophical to suppose that a febrile miasm may be injurious during the first two years of infancy, and that the increased stability and firmness of the constitution may, afterwards, resist its deleterious effects. The second objection involves an assumption of knowledge to which medical science has not yet attained. We do not know the exact periods of time required for the production of different febrile miasms. The miasm of yellow fever requires the continuance of tropical heat, at least for a month.* The miasm of cholera infantum may require a less degree of heat, for a comparatively short period.

Among the concurrent causes of this disease, we may mention the age of the patient, dentition, high atmospheric temperature, impure air, atmospherical vicissitudes, and errors in diet, with premature weaning.

1st. *The Age of the Patient.*—The animal organism is, as yet, in the progress of development. The nervous system is in a state of growth, and undergoing those secret changes of nutrition, by which its organization is to be completed. The process of nutrition causes to be centred, there, a full supply of blood, and a high degree of vascular action. The mucous membranes are undoubtedly in a comparatively imperfect state, in regard to their organization. Their consistence is so soft as to be readily scraped off after death, in the form of a pulp. When their organization is completed, they probably possess sufficient tenacity to be dissected off as distinct membranes. The functions depending, for the regularity of their performance, on the condition of the several structures, are liable, at this period, from comparatively slight causes, to be exalted from a physiological to a pathological state. The

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* Caldwell on Malaria.

vis conservatrix naturæ, the power, which, in the perfect state of the
animal organism, maintains an equilibrium in the distribution of the
vital forces, opposes, in the irritable state of infancy, but a feeble effort
to the action of febrile miasm.

2d. *Dentition* is so marked an agent in the production of this disease,
that some writers have thought it a necessary cause. But the fact that
the cholera infantum occurs, occasionally, after the period of primary
dentition, renders it unphilosophical to consider it in any other view than
as a concurrent cause. Dentition, in some rare cases, causes no general
disturbance of the system, and simply excites an increased secretion of
saliva, and perhaps of the pancreatic fluid. In most cases, it causes a
morbid irritability through the whole frame, and kindles disease in those
structures which are in a state of growth, especially the nervous system
and the mucous membranes. Its chief agency is exhibited in associating
together diseases of the nervous centres with diseases of the alimentary
canal. Hence in cholera infantum it acts by producing a primary cere-
bral irritation, and a consequent irritability of the stomach and bowels.
Constitutional irritation from teething occurs, chiefly, during the period
between the fifth and twentieth month. Hence the prevalence of cholera
infantum during this period of infantile life.

3d. *High Atmospherical Temperature.*—This disease occurs, only,
during the warm season in temperate regions. Its prevalence and fatality
are very marked in warm climates. Dr. Dunglison, in his work on
Hygiene, explains the morbid influence of an elevated temperature on
the animal economy, in the following manner : " The constant evapora-
tion by the cutaneous and pulmonary transpiration maintains the absorb-
ents of the intestines in a state of irregular erethism, which predisposes
them to a morbid condition." High ranges of atmospheric temperature,
without doubt, have a tendency to maintain the functions of the skin, the
liver, and the absorbents of the intestinal canal, particularly the upper
part, in a state of constant excitement. The pulmonary function, also,
is not properly performed in high states of temperature. The blood, not
undergoing its salutary changes in the lungs, becomes a powerful agent of
disease. The morbid matters, retained in the blood from the imperfect
exercise of the depurating organs, are carried, by the vascular system, to
the seats of irritation, established by the concurrent causes of the disease.

4th. *Impure Air.*—The impure air of cities, independent of the spe-
cific miasm, predisposes the system to the disease. It acts through the
medium of respiration, contaminating the blood, and lowering the general
tone of the system. In the narrow lanes and alleys, and in the filthy and

crowded habitations, of our large cities, the morbid agency of impure air is seen in the great prevalence of this disease. Dr. Parrish has well described its effects. " Let any one take a walk, in a summer morning, through the thickly built lanes and alleys of Philadelphia, and he will be struck with the appearance of the children reclining their heads, as if exhausted, upon the breast of their mothers, with a pale and languid countenance, a cool and clammy skin, a shrunk neck, and other signs of debility, arising from their confinement, during the night, to close and hot apartments." The prevalence of an epidemic principle seems to increase the mortality of the disease. Thus, during the prevalence of the malignant cholera, the number of deaths from cholera infantum, in Philadelphia, was as follows, according to the tables of Dr. Emerson.

	June.	July.	August.	Total.
1831.	45	132	82	259
1832.	25	134	157	316

5th. *Atmospherical Vicissitudes.*—The infantile system, exhausted and irritated by heat, dentition, and impure air, is extremely susceptible to the influence of atmospherical impressions. The cutaneous exhalents, debilitated by over-excitement, fall readily into a state of collapse, on the sudden application of cold or moisture; especially at night, during the inaction of sleep. The suppression of the cutaneous function destroys the balance of the circulation, and determines the blood to the internal organs.

6th. *Errors in Diet.*—The digestive mucous membrane, from its delicate, and perhaps imperfect texture, is liable, during the period of dentition, to morbid action. Nature has specially protected it from irritation by two expedients: 1st. A secretion of mucus, which lines the internal surface of the alimentary canal. 2d. The milk of the mother, the blandest and most digestible nourishment. Premature weaning, by substituting an artificial diet for that which Nature has appropriated to the infant, produces febrile disturbance and irritation of the digestive mucous surface. Hence the diarrhœa of teething children often follows weaning at an improper age or season. The following valuable remarks, by Dr. Jackson, are worthy of attention. " Children are benefited by living principally on the breast for twelve months; their vigor is evidently impaired, in almost all cases, when they are nursed less than nine months. The safest period of the year for weaning, is from the middle of October to the middle of March; provided they be not weaned under ten months, after December; under eleven, after January; nor under twelve, after

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February. Children who are weaned at the age of twelve months in March, are ordinarily safe; those who are weaned at this age in April are less so, one half of them suffering severely in the subsequent summer or autumn. In May the danger increases; and in the four subsequent months, if a child of any age be weaned, it will in most cases be very sick before the middle of the October ensuing."* In children, who have been weaned at the improper age and season, food of difficult digestion and overfeeding frequently cause disordered function of the digestive organs.

Pathology.—The pathology of this disease will be inferred from a consideration of the symptoms during life, and an examination of the lesions of structure, in fatal cases, after death. The following appearances were observed by Dr. James Jackson and Dr. John C. Warren, of Boston, from examinations made during a period of several years.

"The body is emaciated; often very much. In some cases the abdomen is full and tense, and especially about the region of the liver. The viscera of the thorax have been found in good order. In the abdomen, the liver has sometimes been found very large, so as to occupy two fifths of that cavity; but this viscus has not presented any other marks of disease, unless, indeed, it may, in one or two cases, have been rather more firm and solid than natural." The gall-bladder, spleen and pancreas, have not been distinguished by any peculiar appearances. "The peritoneal coat of the intestines has, in its greater part, been found healthy; in some cases altogether so; but in most cases some few spots, or portions of it, have been discolored in consequence of a distention of the small vessels going to supply the internal membranes or coats. Also in one or two cases, an inflamed line has appeared on each of two contiguous folds of intestine, just above their line of contact. In every case marks of disease have been discovered on the mucous membrane. In the stomach there have usually been observed one or two small spots, of an irregular shape, in which the mucous membrane was red, inclining a little to a purple. The membrane in these places has not been much, if at all, swollen. The stomach is commonly lined with an adhesive mucus. In the duodenum there have invariably been found one or more spots, much larger than in the stomach, in which the mucous membrane has been considerably inflamed, and for the most part swollen. In almost every case, such an inflamed patch has been found at the very commencement of the duodenum. Other inflamed patches, varying in size, and corres-

ponding with the discolored portions of the peritoneal coat, have been seen in the small intestines in every case."*

Dr. Dewees has found, in the small intestines, coagulable lymph spread over the surface, or in detached pieces. He notices an alteration of structure, from thickening of the coats of the intestine, reducing the calibre of the alimentary canal in the parts where it occurs.

Dr. Horner,† from some careful and accurate post-mortem examinations, infers that cholera infantum is a follicular, rather than an erythemoid inflammation—a disease of the innumerable mucous glands or follicles extended from one end to the other of the alimentary canal, rather than a common vascular or erythemoid inflammation. In the cases examined by him, the stomach was of a sienna color, and of such consistence as to be readily scraped off with the finger nail; the small and large intestines were of the same color, and presented clusters of enlarged and tumid muciparous glands or follicles. The follicles were of the size of millet seeds, and gave to the mucous membrane the appearance of having been sprinkled with fine white sand. By macerating the intestines, and suspending them in spirits of wine, so as to remove the blood and mucus, the anatomical character of the disease was clearly demonstrated to be an ulceration and tumefaction of the follicular system of the intestines. In one case, by maceration and suspension in a fluid, he discovered several common erythemoid ulcerations of the jejunum, of about two lines in diameter, which were imperceptible during the dissection.

Cruvelhier has described a disease, resembling, in symptoms, cholera infantum, under the title of " maladie gastro-intestinale des enfans, avec desorganization gelatriaforme," characterized by excessive thirst, frequent vomiting and purging of mucous and bilious matter, rapid emaciation, and at last an inclination to sleep, from which the patient is roused by abdominal pains, causing plaintive cries, and violent contortions of the body. Fatal collapse often ensued, in the course of twenty-four or forty-five hours. The chief morbid appearance was a gelatinous softening of the stomach, and the small and large intestines. He attributes the pathological alteration to a gastro-intestinal irritation, the special nature of which is unknown. He thus describes the alteration of structure :—
" Ce ramollisement procède toujours de l'intérieur vers l'extérieur. Il y a d'abord simple écartement des fibres, que sépare un mucus gelatineux, et par conséquent les parois de l'organe sont envahies, disparaissent enfin, de telle sorte que l'estomac ou l'intestin ramallis ressemblent à de la

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* New England Journal of Medicine and Surgery, Vol. I. p. 25.
† American Journal of the Medical Sciences, No. VI.

gélatine transparente, arrondie en tube ou en portion de tube.   Si la transformation est complete, les parties désorganisées sont entraînées couche par couche, et ce qui reste paraît aminci ; le péritoine seul résiste quelque temps ; mais enfin, envahi lui-meme, il s'use, se déchire, et la perforation a lieu.   Les parties ainsi transformées sont decolorées, transparentes, d'apparence inorganique, completement depourvues de vaisseaux, exhalant une odeur aigrelette semblable à celle du lait caillé, sans odeur ni de putréfaction ni de gangrène.   Un fait digne d'intérêt, c'est que les parties ramollies se décomposent beaucoup moins promptement que les parties non alterées dans leur organization.   L'ébullition qui convertit en gélatine l'estomac, et les intestins, donne une idée parfaite de ce genre d'altération.   Je dois noter ici un phénomène bien remarquable ; c'est la coloration noire des vaisseaux qui avoisinent l'altération, couleur que je n'ai jamais rencontrée ni dans les parties désorganisées, ni dans les liquides contenus."

The peculiar miasm, which produces cholera infantum, acts upon the minute ramifications of the ganglionic nerves, in the lungs, and by means of the blood throughout the vascular and capillary systems. This primary influence of the miasm on the organic nerves is succeeded by excessive secretory irritation of the follicles of the mucous membrane of the alimentary canal, which constitutes the disease.   The minute and accurate researches of Dr. Horner evince that this disease extends beyond the limits prescribed to it by Dr. Jackson and Dr. Dewees, and that it prevails through the whole extent of the gastro-intestinal mucous membrane. The constitutional disturbance produced by this disease is readily explained by the extent, the relations, and the important functions of the alimentary mucous membrane.   The morbid excitement prevailing through this extensive exhaling surface, causes active determination of the blood to, and profuse secretion of mucus and serum from, the exhalents and follicles.   The functions of digestion, the secretion of the liver, and the processes of nutrition, are suspended.   The evacuations of sero-mucoid fluid by vomiting and purging, produce rapid emaciation, drain the vascular system of the serum of the blood, suspend hæmatosis, and prostrate the vital forces of organic life.

The process of dentition, and the intense irritation of the gastro-intestinal mucous membrane, produce an irritation of the nervous apparatus of animal life.   Hence arise the spasms, the pains, which in severe cases are agonizing, and the convulsions, which precede death so frequently in this disease.   The contrast between the condition of the system of organic life and that of animal life, is beautifully illustrated by Dr. James Jack-

son, in his description of the protracted form of the complaint. "When asleep, the patient is impressed with the characters of death—his countenance deathly, his pulses quick and wiry, his respiration scarcely to be heard; but when he awakes, his clear eye seems to view the objects around him with a peculiar intelligence. With the utmost decision he chooses the pleasant, and rejects the offensive things, which are offered him. He seems almost to tell you, by his actions, that his stomach is faint, and sinking, and distressed; that the call for something to support it is most painfully imperious; but that the appetite can scarcely find an article which does not disgust it. The child is not disposed to make exertions; but when he does, there is often displayed a momentary energy of will, altogether disproportioned to the other appearances about him. He does not express pleasure; and at the most, only assents to what pleases him; but he frets at what disappoints him, and scolds most sharply at what offends him."

The cerebral irritation is very likely to cause congestion, inflammation, and serous effusion. Hence at last the animal powers fail—the patient sinking into a somnolent state, from which he is roused, occasionally, by excruciating pains in the bowels.

In regard to the nature of the disease, we believe it to be situated in the follicular system of the gastro-intestinal mucous membrane. The pathological appearances are various, and the evidence accumulated may not seem sufficient to enable us to separate, with exactness, the accidental from the constant lesions of structure. If this be the case, post-mortem examinations, conducted according to the accurate method of Dr. Horner,[*] cannot fail to establish the true pathology. The following considerations render it highly probable that this disease is seated in the follicular system. 1st. Children are liable to have the follicles of the gastro-intestinal mucous membrane highly developed, which development renders them more susceptible to disease.[†] 2d. This disease, towards its close, affects not only the follicles of the mouth and fauces, but of the cutaneous surface. 3d. A disease of the follicles of the gastro-intestinal mucous membrane, readily accounts for the severity of the constitutional affection, from their immense number.

Treatment.—The indications of cure in this disease, are to allay the irritability of the stomach and bowels, to determine to the surface, to

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[*] We refer to pathological researches, by the aid of minute injections of the diseased membrane.
[†] Dr. Hope.

guard against local inflammation, to support the strength, and restore a healthy tone to the organism.

1st.   The leading feature of this disease is an excessive irritation of the follicles of the gastro-intestinal mucous membrane.   This irritation causes a determination of blood to the digestive mucous membrane, and an exhausting secretion of sero-mucoid fluid.   To allay the irritation of the mucous membrane is, then, an object of the first importance.   A few leeches are to be applied to the epigastrium.   An enema, consisting of a solution of common salt in warm water, is to be administered, and repeated pro re nata ; for a child, a year old, a gill of warm water to a teaspoonful of salt will be the proper proportion.   The injection removes whatever fæcal matter may have collected in the large intestines, and exerts, probably, through the medium of the ganglionic nerves, a salutary effect upon the hepatic secretion, and thereby allays the gastric irritability.   Dr. Dewees has seen this simple remedy frequently relieve the patient, almost entirely without the aid of any other remedy.   The application of leeches to the epigastrium should be succeeded by the repeated application of warm poultices over the abdomen.   If leeches cannot be obtained, other measures of revulsion must be adopted.   The patient may be put into a warm bath, rendered stimulating by adding salt ; the surface may be rubbed, immediately on coming out, with some stimulating liniment.

R. Liquoris Ammon. ℥j.	R. Tinct. Cantharid. ℥iij.
Olei Olivæ ℥ij.	Olei Terebinth. ℥j.
misce benè et adde	Ammoniæ Liq. ℥iss.
Spt. Camphoræ ℥ij.	Saponis Duri ℥j.
Olei Terebinth. ℥iij.	Olei Limonis ℥j.
Saponis Duri ℥v.	M. ft. Linimentum.
misce benè.	
Olei Limonis ℥ij. M.	

The warm bath and the stimulating frictions should be used daily during the continuance of the disease, and may be repeated according to the severity of the gastro-intestinal irritation.   Blisters, applied over the epigastrium, are a valuable means of counter-irritation.   From their occasional severe local effects in infants, they should be applied for two or three hours only at a time, and be followed by the repeated application of emollient poultices.

The vomiting is so severe, in this disease, as often to require the application of particular remedies to allay it.   Dr. Dewees recommends, for this purpose, a teaspoonful of strong coffee, without sugar or milk, to

be given every fifteen minutes. Equal proportions of milk and lime
water, toast water, and *small pieces of ice* (given frequently to children
of sufficient age), may be tried. Hops, the green leaves of the garden-
mint, or green peach tree leaves, steeped in hot water or vinegar and
water, and applied warm, and nearly dry, over the stomach and breast,
will be useful. These remedies, with iced and demulcent drinks, and a
few doses of hydrarg. cum creta, with magnesia or soda, will in many
cases effectually allay the gastro-intestinal irritation. If the severe vom-
iting and purging continue, and an exhausting secretion from the gastro-
intestinal mucous membrane, minute doses of sub mur. hydrarg. and ipe-
cacuanha may be administered.

> R. Sub Mur. Hyd. grs. iij.
> Pulv. Ipecacuanhæ grs. iij.
> Sacch. Alb. grs. xij.
> Ft. Pulveres xij.

One of these powders may be given every half hour or hour, till the
stools evince a decided restoration of the hepatic secretion. The mode
of the operation of calomel, in minute doses, is not to be illustrated by
the principle of direct revulsion; for it not only changes the morbid action
of the follicles, but it excites to a healthy action the hepatic and cutane-
ous secretions.

In addition to this plan, it is important to administer remedies calcu-
lated to give the patient rest during the night, otherwise the pain and
frequent evacuations may produce a fatal exhaustion of the vital forces.
To effect this purpose, it will be proper to place the patient, for eight or
ten minutes, in a bath of a temperature from 90 to 95 degrees Fahren-
heit; the skin should then be wiped dry, and friction employed to excite
the surface. A little paregoric and wine of ipecacuanha may some-
times be given previously to the use of the warm bath. The effects of
opiates should, however, be carefully watched, especially their influence
on the brain. If they have an injurious influence it will be readily seen
on the following morning, in the heavy appearance of the eyes and
countenance, in the dryness of the tongue, and the enfeebled state of the
stomach.

Another indication is to guard against the occurrence of local inflam-
mation.

Cerebral inflammation is a frequent complication of this disease. To
prevent such an occurrence, blisters may be applied to the mastoid
apophyses. Dr. Eberle always applies blisters behind the ears, from the
commencement of the disease. Dr. Parrish says, " in severe cases,

much good may be expected from the application of blisters behind the ears.   I was led to this practice, by observing that the eruption, which, during dentition, is apt to make its appearance behind the ears, often proves a most salutary effort of Nature ; and that while it continues, the infant generally enjoys an exemption from those dangerous disorders incident to this critical period of life.   To imitate nature as closely as possible, the discharge from the blistered surface should be maintained for some time by stimulating dressings.   I have witnessed the most beneficial effects from this practice, and can strongly recommend it to the attention of the profession."   If cerebral irritation be increased by inflamed or swollen gums, they should be freely divided.   If the hepatic secretion be suspended, minute doses of calomel and ipecacuanha should be given.   If the intestinal irritation appear to aggravate the cerebral affection, after a few leeches have been applied to the temples, small doses of Dover's pulv. hydrarg. cum creta, and pulv. antimonialis, may be administered in mucilage of gum arabic.

Acute Enteritis sometimes supervenes in this disease.   When the discharges become bloody, or consist of a muco-sanguinolent fluid, and tenesmus occurs, with other dysenteric symptoms, mucilaginous enemata, with a few drops of laudanum, may be administered.   If the tongue be red, dry, and parched, and tenderness exist on pressure on the abdomen, two or three leeches should be applied along the course of the colon, and afterwards a large emollient poultice over the abdomen.   Dover's pulv. and hyd. cum creta may then be given, as—

> R. Pulv. Dover. iij. grs.
>   Hyd. c. Cret. Əj.
>   Pulv. Gum Arab. Əij.
>   Ft. x. Pulv.

One powder may be given every two or three hours, till the symptoms abate.   Gum arabic water is to be freely given in the mean time. If the sanguineous discharges be profuse, a continuation of opium, ipecacuanha and acet. plumbi will be useful.   When the disease affects more particularly the small intestines, as indicated by vomiting, thirst, a red tongue, diarrhœa, tympanitis and tenderness on pressure, leeches, or a blister to the epigastrium, to be followed by the application of a large emollient poultice, Dover's pulv. and hydrarg. cum creta, and iced demulcent drinks, will be our chief dependence.   Spirits of turpentine has been recommended by different authors as a specific for tympanitis; but clinical experiments have proved this article to be injurious in tympanitis occurring in the early stage of enteritis.   In such cases, the sub-

sidence of the tympanitis from the use of the turpentine is only temporary. It returns, afterwards, in a more aggravated form.

Cholera infantum frequently terminates in chronic diarrhœa. The stomach is very much enfeebled, and incapable of performing its functions. Its irritability increases with its debility, and it rejects, almost immediately, whatever nourishment may be taken. The skin is dry and withered, the patient restless and irritable. The stools vary in appearance, according to the existence of acidity, the state of the hepatic secretion, and the degree of inflammation. If the evacuations be sour, greenish, watery and frothy, alkaline and cretaceous preparations should be employed, as—

> R. Creta ppt. ℥iij. or Carb. Sodæ ℥jss.
> Tinct. Thebaic. gtt. xx.—xxx.
> Ol. Cinnam. gt. j.
> Sacch. Alb. ℥ij.
> Aq. Font. ℥iij.
> M. ft. Julap. (Dewees.)
> s. tea-spoonful every two, three, or four hours.

Dr. Kuhn, of Philadelphia, was in the habit of giving a tea-spoonful of the following mixture every two hours, to correct acidity.

> R. Magnesiæ calcin. ℈iv.
> Pulv. G. Arab. ℈j.
> Sacch. Alb. ℥ij.
> Aq. Menth. ℥ss.
> Aq. Fontanæ ℥ijss.
> M. adde Aq. Ammoniæ, pur. gtt. xlviij. to clxiv. according to the age of the patient.

Preparations of rhubarb will also be useful, from their tonic effect on the stomach and bowels. A tea-spoonful of spiced or simple syrup of rhubarb, combined with a small quantity of laudanum, may be given every three hours till it checks the too frequent discharges. To correct the functional disorder of the liver, one fourth of a grain of calomel, with one half of a grain of Dover's powder, or one twentieth of a grain of opium, may be given every four hours. A few grains of prepared chalk may be added to each powder, to correct the acidity of the primæ viæ. To prevent the too sudden suppression of the discharges, the bowels must be regulated by an occasional dose of castor oil, with a few drops of laudanum.

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When the tongue is dry and coated, or dry, smooth and polished,[*] the discharges black, pitchy, and exhausting, and the skin of a shrivelled appearance, Dr. Cartwright advises a little of the ext. of white walnut, one fifth of a grain of acet. plumbi, and a very minute portion of opium, given every three or four hours. He also uses the croton oil, for the exhausting discharges; one third of a drop, in syrup of roses, may be given to a child a year old, when the abdomen is tense, sore and swollen, and the pulsation of the carotids is quick and weak.

When we have evidence of a tendency to structural changes in the mucous membrane, the stools being slimy, watery, of a red color, and like the washings of flesh, the abdomen tender on pressure, the patient drawing up his legs when lying down, the pulse rapid, and the emaciation general, two or three leeches or a blister may be applied to the abdomen. These measures may be followed by the renewed application of large emollient poultices, and the frequent use of small doses of calomel and opium, or of hyd. cum creta, and sub carbonate of soda, with camphorated tincture of opium, in mucilage of gum arabic.

If the tongue be loaded and the stools slimy, the balsam of copaiva, in doses of five or six drops, or the spirits of turpentine in doses from five to twenty drops, with a drop or two of laudanum, may be given, with benefit, three or four times a day.

When the signs of follicular ulceration are decided, and the stools are mixed with purulent matter, small doses of the chlorate of lime, or of the chloride of soda, may be administered. The nitrate of silver, dissolved in gum arabic water, in doses of half a grain, with one or two drops of laudanum, the sulphate of iron, and the sulphate of copper, in doses of one eighth of a grain, with one twentieth of a grain of opium, are advised by Dr. Eberle, three or four times in a day.

The other indications of this disease are to support the strength, and restore the healthy tone of the organism. In the acute stage of this disease, the debility of all the important functions, especially the vital functions of the respiratory and circulating systems, is caused by an excessive irritation of the innumerable follicles of the intestinal mucous membrane. To remove this prostration, we must not apply to the irritated membrane tonics, stimulants, and astringents, but administer remedies calculated to soothe irritation, and prevent inflammatory action. By applying stimulants and counter-irritants to the skin, we shall allay the secretory irrita-

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[*] When the discharges are acrid, dark-colored and offensive, Dr. Condie gives from five to ten grains of pulverized charcoal, four grains of rhei, and one grain of ipecac., every three or four hours.

tion, and restore the exhausted functions. If the prostration be excessive, in the early stages, frictions, with stimulating liniments, as equal parts of aq. ammoniæ and oil of amber, or fomentations with hot brandy, containing a few pods of red pepper, and the internal administration of a few drops of tincture of cinnamon or a little wine whey, may be resorted to with advantage.

The advanced stages of the complaint are more adapted to the use of tonics, stimulants, and astringents. For severe colic pains, from flatulent distention of the stomach and bowels, Dr. Eberle uses from ten to fifteen drops of the following solution, three or four times daily.

R. Ol. Juniper 3ij.
Sulph. Æther 3ss.
Tinct. Opii gtt. lx.
M. ft.

When the hepatic secretion has become healthy, astringents and tonics will be of service to restore the tone of the intestinal mucous membrane. For this purpose we may use a decoction of blackberry root, or of geranium maculatum, in milk, or of pomegranate bark and flowers; or an infusion of columbo root, or of logwood; or a combination of chalk mixture with tinct. of kino, or sulphate of quinine in syrup of roses. Dr. Chapman uses the supersaturated sulphate of iron.

R. Sal Martis gr. ij.
Acid Sulph. Dil. gtt. x.
Sacch. Alb. 3j.
Aq. Font. 3j.
M. 3j. pro dosi.

Dr. Eberle has found a mixture of equal parts of lime water and infusion of Peruvian bark most beneficial in restoring the tone of the alimentary canal. He gives a dessert spoonful of this mixture, with four or five drops of tinct. of kino, in a solution of gum arabic, four or five times daily. During convalescence, the abdomen should be bound in a flannel roller.

The diet must consist almost exclusively of breast milk, in infants under the age of a twelve month, or who have been recently weaned. A healthy wet nurse should always be procured for children who have been weaned at an improper age or season. Gum arabic water may be given, occasionally, where the child is affected with excessive thirst. In

children who have been weaned, or who will not nurse, barley or rice water and milk, toast water, gum arabic water, soda water, marshmallow tea, infusion of toasted oatmeal, and liquid farinaceous preparations of arrow-root, tapioca, sago, rice and boiled flour, will constitute proper articles of nourishment. In the protracted form of the disease, beef tea, chicken tea, and animal broths, will be of service. Dr. Rush advises a more stimulating diet, as salted meats, where the child has an instinctive craving for them. A removal from the city to the country, or to the sea side, exerts a most salutary effect upon this disease. A change of air cannot be too highly appreciated as a means of cure. If the advantage of a removal from the town to the country cannot be enjoyed, the child's residence may be changed from a low and moist to a high and dry situation, and he may be daily exposed to the fresh air, either by being carried out by means of attendants, or by frequent rides into the country. When the patient has been restored by a removal to the country, he should not be returned to the city until the middle of October, or while the miasm of cholera infantum continues to prevail.

Prophylaxis.—The prophylactic measures consist in protecting the infant from the action of the specific miasm, and in guarding it against the effects of the concurrent causes of the disease.

First, the specific miasm. Dr. Caldwell has suggested, in his dissertation on malaria,* the following preventive measures.

1. The best and only certain means of protecting infants from cholera infantum, is to allow them to pass their summer in the country. 2. The next best plan of security, is to allow the patient to sleep in the country every night, during the summer months; because exposure to the miasm, at night, during the inaction of sleep, is more dangerous than exposure during the day. 3. Where these measures are not convenient, the child should pass his nights and days, when at home, in the upper stories; because the febrile miasm does not rise to the highest stories of lofty city dwellings, or, at least, does not reach them in a state of full concentration and strength. 4. A few hours exercise daily, in the open air, especially in the country, without the limits of the malaria, will contribute to maintain the vigor of the system, and to protect it from the disease. The coolness of evening, and the extreme heat of noon-time, should, however, be avoided. 5. An artificial eruption on the skin, by maintaining a centrifugal action, would probably protect the system from the

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* American Journal of Medical Sciences, No. xvi., 1831, p. 330.

influence of the miasm. Children affected with prickly heat escape cholera infantum, unless from sudden change of the atmosphere, or other cause, the eruption disappears.

Beside these means, the child should be clothed in flannel, and the lower extremities kept warm by the use of worsted stockings. The frequent immersion of the child in cool water, and the use of the tepid bath, will promote cleanliness, invigorate the system, and render it less susceptible to the action of the miasm.

It is important that the child be cool and comfortable during sleep. The child's bed should consist of a mattress, or of folded blankets laid on the floor, and light covering. The air of his sleeping apartment should be rendered cool, and as pure as possible; the door of the room being kept open, and the windows, with the shutters closed, if he sleep in the upper stories.

Dr. Parrish recommends the free use of cool and fresh water, during the heat of summer; and in infants predisposed to the disease, moderate quantities of weak infusions of ginger, nutmeg and cinnamon. The tone of the stomach, in languid infants, is raised by the moderate use of spices, pepper, cloves, and the sucking of small pieces of salt meat, as ham or dried beef.

Dr. Rush advises the use of sound old wine in the summer months. "From a tea-spoonful to half a wine-glassful, according to the age of the child, may be given every day. It is remarkable that the children of persons in easy circumstances, who sip occasionally, with their parents, the remains of a glass of wine after dinner, are much less subject to this disorder than the children of poor people, who are without the benefit of that article of diet." Dr. Eberle has found the use of small portions of porter and water beneficial in feeble and relaxed infants, as a preventive to bowel complaints. Farinaceous preparations of arrow-root, sago and tapioca, and weak animal broths, form the best nourishment for children who have been weaned. The occasional use of a moderate quantity of salted meat is advised by Dr. Rush. In children who have not been weaned, healthy breast milk must constitute the chief nourishment.

Other important prophylactic remedies will now be enumerated, as necessary to guard the infant against the ill effects of *dentition.* The preventive measures are:—1st. Exercise in the open air. 2d. Daily cold sponging, followed by friction. 3d. Particular attention to produce coolness of the head; washing the head, daily, with cold water. 4th. Proper regulation of the diet. The nurse should avoid stimulants in her

food and drinks. The child should take the breast often, but not long at a time, to prevent overfeeding. 5th. Attention to the state of the gums. Painful tension should be relieved by a free incision of the gum and capsule; and if the advancing tooth be double, a crucial incision should be preferred. 6th. Gentle laxatives, when plethora exists, or where the customary salivation is not present. 7th. Blisters, or the occasional application of one or two leeches behind the ears, if there be determination of blood to the head. 8th. Avoid premature weaning, as within the year, or weaning at an improper season, as between the months of May and October.

THE STABILITY OF SOLID CALCIUM
HYPOCHLORITE

BY

MAJOR H. H. KING, I.M.S.
Central Research Institute, Kasauli

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THE war brought into prominence the use of hypochlorites as disinfectants, first for the sterilization of drinking water, and secondly, either alone or with other substances, for surgical purposes. Later years have seen their use extended, so that now they hold a position of the greatest importance. The hypochlorites mainly used are bleaching powder and substances derived from it. Solutions of sodium or calcium hypochlorite chemically or electrolyticly prepared are also used. Here, I wish to bring to the notice of the English-speaking world generally, a substance, solid calcium hypochlorite, whose advantages, as regards concentration and stability, seem to be appreciated at present most in Germany. By calcium hypochlorite I mean the salt in which *both valencies* of the divalent calcium atom are satisfied by hypochlorite radicles, and which would thus have the formula $Ca < ^{OCl}_{OCl}$. Now, whatever may be the real constitution of bleaching powder, there is no doubt that quantitatively its formula is fairly accurately expressed by $Ca(OCl)Cl$ plus a varying amount of lime. Therefore it is obvious that theoretically solid calcium hypochlorite can have almost double the available chlorine strength of bleaching powder, and more than double, if there be but little free lime present with the calcium hypochlorite as compared with the 33 per cent or more present in 'bleach.' To turn aside for a moment, I need hardly point out that the *available* chlorine is not necessarily the same as the *actual* chlorine present. The available chlorine is the amount of free chlorine to which a substance is chemically equivalent. Thus one atom of chlorine in a hypochlorite radicle is equivalent to two atoms of free chlorine. To return to calcium hypochlorite, theoretically it promised so much if only it were stable, that it seemed well worth investigation. Thorpe's Dictionary of Applied Chemistry stated that it was manufactured in Germany under certain patents, and described it as a crystalline solid that contained 80 to 90 per cent of available chlorine, and that it was reputed to be stable.

Now it is well known that, for all practical purposes, the disinfectant action of hypochlorites is proportionate to their available chlorine strength, so these my first experiments, which I shall describe, have been devoted to ascertaining whether the promise of high and sustained available chlorine strength is fulfilled in practice. I would recommend those who are interested in its direct disinfectant action to read a paper by Fischer and Kadisch in the Zeitschrift für Hygiene, April 1924.

Tins of the calcium hypochlorite preparation were posted to me from Manchester at the end of November 1923, and reached me at Kasauli in January 1924. Two tins tested in February were found to have the same available chlorine strength of 74·4 per cent—their original strength must have been higher still—the substance is made in Germany. In order to test the effects of (A) ordinary storage in the dark, (B) heat, (C) light, (D) vibration, (E) exposure to air, the following experiments were made. Five small glass stoppered bottles were half filled with calcium hypochlorite and the stoppers waxed down. The first was put in a wood box which was kept in an ordinary room cupboard. The second was put in an incubator (in the dark) at 37°C. (i.e., at 98°F.) for 66 days, after which it was kept in a box in a cupboard for 174 days from May to October ; then it was put back in the incubator at 37°C. for 67 days, so that it was exposed to a temperature of 37°C. for more than 4 months, and to the air temperatures of a hill hot weather and monsoon for a little more than 6 months. The third bottle was put on a shelf exposed to indirect light. The fourth was put in a wood case and shaken by a vaccine shaking machine for about six hours a day on 12 days in the first month after which it was kept in the cupboard. The fifth was put in a wood case open at the top and was left unstoppered on a shelf. At the end of the 10 months the final readings for available chlorine strength were as follows:—

(A)	Cupboard (in the dark)	.. .. ..	§ 67·7 per cent.
(B)	Incubator 4 months, cupboard 6 months	..	56 ,,
(C)	Exposed to light ..	.. .. ..	66·7 ,,
(D)	Exposed to vibration	.. .. ..	67·7 ,,
(E)	Exposed to the air ..	.. .. ..	24 ,,

The initial strength was 74·4 per cent, so clearly ordinary storage did not seriously affect the chlorine strength nor did light or the small amount of early vibration. The only factors that had any force were heat and exposure to the air. The latter need not concern us, as it is not legitimate treatment. What, indeed, surprised me, seeing the sticky mass that was left, was that the chlorine reading was as *high* as 24 per cent. Though the effect of heat on the half full bottle was to reduce the strength from 74·4 per cent to 56 per cent, yet its effect on a full tin, treated in exactly the same way, was only to reduce the strength to 62·3 per cent, so that the claim made by its manufacturer that the minimum strength is 60 per cent held good for a full tin. Tested after the first 2 months in the incubator, the strength of both the tin and the bottle was 67·4 per cent, which is a comparatively high figure. Seeing that it is very unlikely that the conditions of storage, so far as the effect of temperature is concerned, in any station in India over 10 consecutive months would be more severe in total effect than the conditions of the

second experiment, we may say that the deterioration there found probably represents the maximum deterioration likely to be obtained anywhere in India in 10 months. In estimating the severity of an incubator temperature we must bear in mind its continuous nature as compared with the short duration of the higher temperatures reached in many stations in the hot weather. Nowhere in India does the monthly mean daily temperature exceed 98°F. My conclusion from these first experiments, is that the samples obtained of solid calcium hypochlorite were reasonably stable.

Before going further, it will be interesting to compare with the results already recorded, the results of exactly similar tests made on ordinary 'bleach' and a 'bleach' stabilized by the addition of lime that is stocked by the official medical stores.

A jar of stabilized bleach of unknown age, tested in February 1924, had an available chlorine strength of 17·7 *per cent.*

Final readings for the same series of five bottles are as follows :—

(A) Cupboard	..	..	..	16·1 per cent.
(B) Incubator and cupboard	..	..	..	12·5 ,,
(C) Exposed to light	..	..	..	15·7 ,,
(D) Exposed to vibration	..	..	..	15·7 ,,
(E) Exposed to the air ..	..	..	..	8·6 ,,

These results correspond more or less closely with the previous ones if we calculate the proportion each loss bears to the initial strength, but of course in this second case the action has been at a much lower level. The small effect of light and vibration, and the great effect of exposure to the air that were noted in the first set of experiments, are also to be noted in this second set. The effect of only 2 months' incubator temperature was to reduce the strength to 15·2 per cent available chlorine.

A bottle of ordinary bleach at the beginning of the 10 months' experiment had a strength of 19 per cent of available chlorine. Two bottles put up for experiments A and B gave the following results :—

(A) Cupboard 10 months	..	..	..	8·3 per cent.
(B) Incubator 2 months	..	..	..	10·5 ,,
(C) Incubator 4 months, cupboard 6 months ..		..	..	0·07 ,,

The instability of ordinary bleach is well shown by these results, particularly the last, with its reading of practically zero per cent. By comparison, both calcium hypochlorite and stabilized bleach seem very stable substances. I think that there can be very little doubt that the chief reason for the superior stability of these two substances is their *dryness.* The samples of calcium hypochlorite obtained were in the form of a very dry powder. A thorough *in vacuo* drying is probably essential for its stability. I would also draw attention to the nature of its containers, first, the tins are *airtight* through the use of a washer round the lids, and secondly, the tins are *painted* with some bituminous paint, thus preventing the deleterious catalytic action of rust.

Apart from its high content of available chlorine, this preparation of calcium hypochlorite has a distinct advantage over bleaching powders in that the proportion

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of free lime and extraneous matter is very low so that a solution in water gives *very little sediment*. This property is of particular value in the chlorination of drinking water.

Given the stability and strength that I have shown to exist in the samples tested, the advantages of preparations of solid calcium hypochlorite as disinfectants are very great. This is particularly the case where transport is a consideration, as in armies on campaigns or manœuvres, in exploring and other expeditions, and in travel generally. Indeed, to my mind, solid dry calcium hypochlorite is the most important of the disinfectants that an army in the field should carry, particularly in frontier and similar campaigns, for not only would it serve for the sterilization of water, but, it would also serve as a surgical disinfectant, both by itself in simple solution and as a source of hypochlorite for the preparation of eusol, etc. The fact that on the average it is three times as strong as 'bleach' would mean that only one-third of the weight of equivalent bleach would need to be carried. It would be an advantage for ready use were it available in the form of tablets. Another situation where it would be of particular advantage is in epidemics of cholera, etc., in districts with bad communications as are common in India.

In ordinary civil life the choice of a hypochlorite disinfectant is governed mainly by the cost. Into this I will not go, as the problem varies with every different locality, except to say that, where electric power is available in India, electrolytic hypochlorite will probably always be the cheapest. My intention here has been to show that dry solid calcium hypochlorite is a stable product, and that it has great advantages as a disinfectant, where portability is a factor of importance.

Finally, there is another use of it to which I may draw attention, namely, its use as a chemical reagent. It is a powerful oxidizing and chlorinating agent and may serve to facilitate certain reactions such as the production of chloramines. I must warn users of it that there is the danger of an explosion if it be rubbed up with organic or other easily oxidized (or easily chlorinated) substances.

There is nothing particular to mention about the technique of the tests for available chlorine, which were done in the ordinary way by titration with deci-normal sodium thiosulphate after the addition of potassium iodide and acetic acid to measured volumes of a known dilution of the bleach. The sodium thiosulphate solution was standardized against a solution of arsenious oxide in sodium carbonate, each being titrated against the same iodine solution. I found it advisable in setting up standards to begin with iodine, which was purified by precipitation and sublimation, and not to begin with arsenic, with which an insoluble residue was repeatedly obtained. Since the iodine solution did not keep well, the arsenic was kept as the permanent standard. In the course of some previous work I happened to observe that the blue 'iodide' of starch disappears when excess of starch is added—indeed the delicacy of the starch test is spoilt if an excess of starch be present. I do not know whether this observation has been made before—I can find no record of it.

A Comparative Study of the Birth Mortality in the Albino Rat and in Man

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

A COMPARATIVE STUDY OF THE BIRTH MORTALITY IN THE ALBINO RAT AND IN MAN

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Literature dealing with birth statistics for man contains numerous references both to the number and to the sex of the stillborn; but in none of this literature, nor in any of the many works covering various phases of animal breeding, is there any detailed information regarding birth mortality in other mammals. It has seemed worth while, therefore, to record the data for stillbirths in the albino rat that have been collected in the course of an extensive series of breeding experiments carried on in the animal colony of The Wistar Institute of Anatomy and Biology. Life processes in the rat accord in many ways with those for man, as Donaldson ('06, '08, '18) has pointed out, and the data given in the present paper indicate that there is also a close agreement in their normal sex ratio, in their birth mortality, and in the sex proportions of the stillborn.

The collection of data regarding the birth mortality in the albino rat was begun June 1, 1913, and carried on uninterruptedly for four years; it was resumed the beginning of June, 1918, and discontinued June 1, 1919. In any investigation of this character it is essential that all of the individuals in a given litter should be recorded, if the data are to have much statistical value. This necessitates, in the case of the rat, an examination of the litters at birth, or shortly after, since stillborn young left for a longer time in the nest are sometimes destroyed. While it is known that the data for the great majority of litters included in this study are complete, there is a probability that some of the stillborn young were omitted from the records, since all litters could not be examined at or close to the time of birth. The magnitude of the probable error is not great enough, however, to affect

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the general conclusions that have been drawn, though more exact data might change somewhat the various percentages given.

Normally, young rats begin suckling soon after their birth, and as at this time and for some days afterward the skin over the abdomen is semi-transparent, any milk in the intestinal tract is readily seen and is a sure indication that the individual was alive when born. There is, therefore, no difficulty in distinguishing the stillborn individuals from those that died later, even though the litter is not examined until a day or two after it is cast. Throughout this paper the word 'stillborn' is applied only to young rats that lived through the normal gestation period (twenty-one to twenty-three days) and died shortly before or during birth, and the mortality data given are for such individuals only. Only a very few cases of abortion have been found in the course of breeding experiments with the rat extending over a period of eleven years and comprising many thousands of individuals; none of these are included in the present study.

THE NORMAL PERCENTAGE OF STILLBIRTHS

During the period in which the birth mortality statistics for the albino rat were being collected, a total of 253 litters were found in which one or more individuals were stillborn. Data for these litters, arranged according to the year in which the records were taken, are given in table 1.

The data given in table 1 show that there was considerable variation in the number of stillbirths occurring in different years. Such variation was to be expected, since the total litter production in the colony varied greatly from year to year (table 2). The percentage of stillbirths in the total number of individuals involved, however, is remarkably constant for all sets of data, as the range of variation is from 20 to 26.2 per cent only (table 1). Since in each year that records were taken at least one-fifth of the young in a considerable number of litters were dead at birth, it is evident that the mortality was not due to chance, but to some disturbance in the metabolism of the mother that tended to involve the litter as a whole.

In order to show the normal percentage of stillbirths in the entire colony, data for the total litter production during the period that the mortality records were taken are given in table 2.

TABLE 1

Data for living and for stillborn young in 253 litters of albino rats. Groups arranged according to the year in which the records were taken

YEAR	NUMBER OF LITTERS	TOTAL NUMBER OF YOUNG	LIVING YOUNG			STILLBORN YOUNG			
			Males	Females	Number of males to 100 females.	Males	Females	Number of males to 100 females	Per cent stillborn in total number of young
1913–1914	29	225	81	99	81.8	25	20	125.0	20.0
1914–1915	78	570	222	225	98.7	70	53	132.1	21.6
1915–1916	35	289	121	104	116.3	36	28	128.6	22.1
1916–1917	51	339	135	115	117.4	48	41	117.1	26.2
1918–1919	60	394	152	148	102.8	55	39	141.0	23.8
	253	1,817	711	691	102.9	234	181	129.3	22.8

TABLE 2

Showing the total number of individuals, including the stillborn, that were produced in a colony of albino rats during a period of five years

YEAR	TOTAL NUMBER OF LITTERS	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES	NUMBER OF STILLBORN	PER CENT STILLBORN
1913–1914	899	6,677	3,379	3,298	102.5	45	0.67
1914–1915	945	7,065	3,690	3,375	109.3	123	1.74
1915–1916	828	6,443	3,273	3,170	103.2	64	0.99
1916–1917	1,023	7,131	3,597	3,534	101.8	89	1.25
1918–1919	625	4,354	2,217	2,137	103.7	94	2.16
	4,320	31,670	16,156	15,514	104.1	415	1 31

As table 2 shows, from 600 to 1000 litters of albino rats were born each year during the period covered by the investigation. The percentage of stillbirths in the total litter production varied considerably in different years, and for the 31,670 births was 1.31 per cent. Assuming, for reasons to be given later, that at most only 8 per cent of the stillbirths that occurred were not recorded, it would appear that the normal birth mortality in the colony, under existing conditions of environment and of nutrition, was not greater than 2 per cent.

During the past seventy-five years an extensive literature has appeared dealing with the birth statistics for man in various countries of the world. From the data given in this literature, Nichols ('07) has compiled a table showing the living and the stillbirths throughout the world during the period from 1751 to 1903. In the enormous total of 447,019,579 births recorded, 13,635,986, or 3.04 per cent, were stillbirths. From a statistical standpoint the various sets of data used by Nichols are not of uniform value, since the laws regarding the registration of births vary greatly not only in different countries, but in different sections of the same country (as in the United States), and therefore many of the records are known to be incomplete. The records of stillbirths, especially, are very faulty, partly because in many countries their registration is not required and partly because the data obtained include fetuses aborted at various stages of gestation. With reference to human births, it may be noted, the word 'stillborn' is used to designate fetuses that are at least seven months of age when born; fetuses aborted at earlier stage of development are not ordinarily included in birth statistics.

More recent series of statistics show a birth mortality for man varying but slightly from that given by Nichols. Thus Auerbach ('12) states that in over 100,000 births as registered in Budapest, 3.3 per cent were stillbirths, and Terry ('17) has shown that in a total of 449,744 births recorded in Massachusetts during 1910 to 1914 there were 15,911, or 3.2 per cent, of stillbirths.

In certain countries in which the laws regarding the registration of births are relatively strict, the percentage of stillbirths is somewhat higher than that given by Nichols. For example, statistics for Prussia during the period from 1872 to 1881 show, according to Düsing ('84), that the stillbirths formed 4.67 per cent of the total of 10,577,478 births; birth statistics for the United States during 1918 show 3.56 per cent of stillbirths in a total of 1,412,283 births (Davis, '20).

The data for human births in one year in selected cities of the United States, as collected by The Children's Bureau of the U.S. Department of Labor, form a unique and valuable series (Duke, '15; Duncan and Duke, '17; Allen, '19; Dempsey, '19). Although

the number of births recorded is very small when contrasted with the large numbers given above, the great care taken to make these records as complete as possible gives to the data great statistical value.

Birth statistics for four selected cities, as collected by The Children's Bureau, are given in table 3.

TABLE 3

Summary of human births in one year based on data collected by the U. S. Children's Bureau in selected cities of the United States. 1, Brockton, Mass.; 2, Johnstown, Pa.; 3, Manchester, N. H.; 4, Saginaw, Mich.[1]

	TOTAL NUMBER OF BIRTHS	NUMBER OF MALES TO 100 FEMALES	LIVING YOUNG				STILLBORN YOUNG				
			Number of individuals	♂	♀	Number of males to 100 females	Number of individuals	♂	♀	Number of males to 100 females	Per cent stillborn in total number of young
1	1,247	105.77	1,210	623	587	106.13	37	18	19	94.73	2.96
2	1,551	110.16	1,463	761	702	108.40	88	52	36	144.44	5.67
3	1,643	101.10	1,564	781	783	99.74	79	45	34	132.35	4.80
4	1,015	107.99	981	507	474	106.92	34	20	14	142.85	3.34
	5,456	105.96	5,218	2,672	2,546	104.94	238	135	103	131.06	4.36

[1] Statistics of births during one year in a fifth city, Waterbury, Conn., have also been published by the Children's Bureau (Hunter, '18). The data given show a total of 2,654 births of which 3.2 per cent were stillbirths. Since sex data are given for only 53 of the 86 stillborn young, this series of data is excluded from table 3.

As shown in table 3, the percentage of stillbirths in the various cities concerned varied from 2.96 (Brocton) to 5.67 (Johnstown), and in the total of 5,456 births recorded there were 238, or 4.36 per cent, of stillbirths. In this series, therefore, the birth mortality is considerably higher than that in any series of data previously cited, yet in each of the papers in which the birth statistics are given it is stated that the number of stillbirths recorded is probably too low, owing to the great difficulty experienced in obtaining accurate information regarding such births. It has been estimated that at least 5 per cent of stillbirths are never recorded, even in localities in which the laws regarding their registration are most rigidly enforced.

Although the birth mortality in domestic animals would seem to be a matter of considerable importance to the stock breeder, there are only a few scattered references to it in works dealing with various phases of stock breeding, and practically no data having statistical value are available for analysis. According to Bernoulli ('41), records for Europe covering a period of ten years show that from 10 to 15 per cent of calves were dead at birth. As, however, a very great proportion of these deaths were undoubtedly abortions due to infectious disease, the normal birth mortality among full-term fetuses is yet to be determined.

Fairly complete records regarding living foals have been kept in various studs throughout Europe for many years, but data for the stillborn are very meager. Records compiled by Hoffman ('85) show that in a total of 1,556 foals, 87, or 5.6 per cent, were stillborn: Goehlert ('84), quoting Baumeister, states that on the average 6 per cent of all foals are born dead: 4 per cent of these are cases of abortion and 2 per cent are of foals at the end of term. In neither of these papers are any data given that show the sex proportions among the stillborn young.

Available evidence thus seems to indicate that in the higher mammals from 2 to 4 per cent of full-term fetuses are dead at birth, and it is probable that at least half of this mortality is due either to disease or to mechanical injury at birth.

THE SEX RATIO IN STILLBORN YOUNG

It is a matter of considerable interest whether the sex ratio, i.e., the number of males to each 100 females, in the stillborn is the same as that in the living young. For if there is a pronounced and constant difference between these two ratios, there must be some disparity between the sexes that is an important factor in the birth mortality.

In order to make possible a comparison between the sex ratio for the living and that for the stillborn young of the albino rat, it is necessary to ascertain the sex ratio that is normal for the species. Cuénot ('99) gives 105.6 males to 100 females as the sex ratio in thirty litters of albino rats; records for over 1000 litters of stock Albinos, as collected by King and Stotsenburg ('15),

show a sex ratio of 107.5 males to 100 females; while the data as given in table 2 of the present paper indicate a sex ratio of 104.1 males to 100 females in a total of 31,670 births.

The normal sex ratio for any species can be obtained only by ascertaining the sex proportions among all of the offspring of a considerable number of females kept under favorable conditions of environment and of nutrition during the entire period of their reproductive activity. None of the sex ratios for the albino rat as given above can, therefore, properly be taken as the norm, since none of them are based on completes series of data. Records covering the complete breeding history of a number of stock albino females have recently been obtained, however, and they show that the sex ratio in the newborn, including those dead at birth, is about 107 males to 100 females. This ratio, therefore, is the one that will be taken as the norm for comparison with the sex ratio in the stillborn.

On referring to table 1 it is found that in each year that mortality data for the albino rat were recorded there was a very great excess of males among the stillborn. While the number of stillbirths in any year was relatively small, the fact that in each set of records the sex ratio varies from the norm in the same direction and to a very considerable degree adds materially to the value of the data. In the total of 415 stillbirths recorded the sex ratio was 129.3 males to 100 females. This ratio is 26 points above that for the living young in the litters concerned, and as it is 22 points higher than the sex ratio taken as the norm (107 males to 100 females) the deviation is much too great to be considered as within the limits of normal variation. Granting that the records for stillbirths are incomplete, there is no reason to suppose that the sex ratio in the unrecorded stillborn would differ materially from that for the recorded stillborn as given in table 1. The evidence at hand, therefore, indicates that in the rat the mortality at birth is far greater among the male than among the female young.

According to Rauber ('00), as early as the year 1660 Graunt showed that more boys than girls were born in the city of London, and this finding has been confirmed by practically every collector

of human birth statistics since that time. Nichols' very comprehensive table of birth statistics, to which reference has already been made, shows a sex ratio of 105.5 males to 100 females in over four hundred million living births. More recent data give practically this same ratio: thus data for 171,297 living births of white and colored children in Cuba during the period from 1904 to 1906, as given by Heape ('09), show a ratio of 105.46 males to 100 females; while among the 465,655 births in Massachusetts from 1910 to 1914 the sex ratio is 105.41 males to 100 females (Terry, '17). Statistical evidence from many different sources thus seems to warrant the conclusion that in all civilized countries of the world there is an excess of males among the living young; the ratio which may be considered as the norm being about 105.5 males to 100 females. This ratio, as several investigators have pointed out, is remarkably constant and is maintained "through periods of war and of peace, of famine and of plenty, and under a great variety of racial and of climatic conditions; the variations, as a rule, being not greater than one per cent" (Pike, '07).

Available statistics for the sex of stillborn children are admittedly very incomplete, yet millions of such births have been recorded and they invariably show a fairly uniform sex ratio that differs in a marked degree from the sex ratio which is the norm for the living young. A few series of investigations may be cited to indicate the trend of such statistics in general. In the 13,635,986 stillbirths compiled by Nichols there were 131.6 males to each 100 females, the range of variation in the number of males being from 130 to 140 in the great majority of cases. In the records for stillbirths in various countries of Europe, as tabulated by Lewis and Lewis ('06), the number of males to 100 females varies from 120 to 170, with the average around 130; Heape's ('09) data for Cuban births shows a sex ratio of 144.45 males to 100 females among the stillborn, while Hirsch ('13) gives 127.9 as the number of males to 100 females in the stillborn young recorded in Germany during 1908 to 1909; and, finally, the birth statistics of the United States for the year 1918 indicate a sex ratio of 137.1 males to 100 females among the stillborn (Davis, '20).

All of the various series of records given above show that the sex ratio in the stillborn is much higher than that in the living young, and sex statistics for aborted fetuses indicate that the excess of boys becomes greater the earlier the month of pregnancy in which the fetus dies (Rauber, '00; Nichols, '07; Auerbach, '12; Carvallo, '12). This latter fact is of great importance, since it indicates that one, at least, of the chief causes for the excessive mortality among males at birth must be sought in conditions that exist in early rather than in late stages of gestation.

In 1841 Bernoulli called attention to the fact that the sex ratio at birth is not the ratio in which the young are conceived, and he concluded that the true sex ratio for man is about 108.2 males to 100 females. This ratio is practically the same as the 'primary' sex ratio recently calculated by Jendrassik ('11) and by Schultz ('18). The fact that in man the sexes are very evidently not conceived in equal numbers is a decided stumbling-block in the way of any theory of sex determination that postulates chance as the chief factor in deciding whether a given ovum shall become male or female. Morgan ('19) has recently offered the following explanation for the constant sex ratio in man:

Since male babies die oftener than females, the difference has been said to be an 'adaptation,' with the implication that it calls for no further explanation. Several possible solutions suggest themselves. The male-producing sperm bearing the sex-chromosome may more frequently develop abnormally than the female-producing sperm. Again, since the spermatozoa must, by their own activity, travel the entire length of the oviduct to reach the egg as it enters the tube, the greater size or weight of the female-producing sperm may give a slight advantage to the male-producing sperm in the long trip up the tube. This would lead to an excess of males.

Since there is no evidence at present that one kind of spermatozoa is more active or more inclined to be abnormal than the other, it must be admitted that the above explanation for the male excess in human offspring is not an entirely satisfactory one.

A comparison of the sex ratios found in the newborn of the rat with the corresponding ones for man show that they agree closely in all cases. The sex ratio that is normal for the living young at birth is practically the same in both species, being about 105.5

males to 100 females; in both species, also, the sex ratio in the stillborn is much higher than that in the living young, averaging about 130 males to 100 females.   This striking similarity in the sex ratios of two such widely separated mammals as the rat and man is a matter of considerable theoretical interest, and it may have a practical bearing as well, since through carefully controlled experiments on the lower form it may be possible to obtain information that will help to check the appalling birth mortality among human offspring.

Although a considerable body of statistics has been collected by Düsing ('84) and by Wilckens ('86), among others, regarding the normal sex ratio in domestic animals, practically no information is available concerning the sex proportions in the stillborn. In fact, the only reference to this subject that I have been able to find is in a paper by Goehlert ('82) which deals chiefly with the inheritance of coat color in the horse.   Goehlert states that in 135,826 living foals born in various studs throughout Europe the sex ratio was 96.57 males to 100 females.   Then follows this significant statement: "Derselbe steigert sich bei den todtgeborenen auf 106 bis 107 Hengst gegen 100 Stutenfohlen."   The data on which the above statement is based are not given, but if they are extensive and accurate enough to have statistical value, they indicate that the sex ratio in stillborn foals is some 10 points higher than that in living foals.   Thus in man, in the rat, and in the horse, the only mammals for which data are at present available, the birth mortality is apparently far greater among the male than among the female young.   It is not improbable that future investigations will show that this condition is characteristic of the Mammalia generally.

SEASONAL VARIATIONS IN THE PERCENTAGE OF STILLBIRTHS

It has been claimed by Düsing ('84) that seasonal variations in temperature, through their action on nutritive conditions, affect not only the sex of developing fetuses, but the percentage of stillbirths as well.

The desirability of recording data for stillborn rats according to the month of the year in which birth occurred was not realized

when this investigation was begun, consequently only the data collected during the last year can be grouped by seasons as shown in table 4.

Stillbirths were recorded in the colony during every month of the year, the smallest number (3) being found in May, the largest (14) in September. On grouping the data as shown in table 4, it is seen that the 94 stillbirths were very evenly distributed throughout the different seasons, the variation in number being from 21 (spring) to 25 (autumn). The percentage of stillbirths in the total litter production, however, shows a wide range of variation in different seasons, being nearly twice as great in

TABLE 4

Showing the percentage of stillbirths in the albino rat colony from June 1, 1918, to June 1, 1919. Data arranged according to the season of the year in which birth occurred

SEASON	TOTAL NUMBER OF LITTERS	TOTAL NUMBER OF YOUNG	NUMBER OF STILLBIRTHS	PERCENTAGE STILLBIRTHS IN TOTAL NUMBER OF YOUNG
Spring (March to May)............	183	1,349	24	1.78
Summer (June to August).........	166	1,134	21	1.85
Autumn (September to November)..	120	821	25	3.04
Winter (December to February)....	156	1,050	24	2.28
	625	4,354	94	2.16

autumn (3.04) as in summer (1.78). The data given in table 4 are, of course, too few to have much statistical value, but they seem to indicate that the percentage of stillbirths tends to vary somewhat with the season, reaching its highest point in the autumn months. Lacking adequate means of heat regulation, rats suffer severely from high temperature, and the young born late in summer and in the autumn are, as a rule, inferior to those born at other seasons as regards their power of growth, resistance to disease, fertility, and longevity. It is not surprising, therefore, to find that this lowering of the physical tone of the animals at a definite season of the year is followed by an increase in the birth mortality.

From an analysis of the data for over ten million births occurring in Prussia from 1872 to 1881, Düsing ('84) concludes that "bei den Kindern, welche im Anfang des Jahres erzeugt und im Herbst geboren werden, zeigen sich die wenigsten (3.6 per cent), dagegen bei denen, welche im Frühjahr gezeugt und in Winter geboren werden, die meisten tot-geburten (4.4 per cent)."   Other groups of statistics for human births do not support Düsing's conclusions, however,   Thus, data compiled by Davis ('20) show that in the birth registration area of the United States during 1918 the lowest percentage of stillbirths occurred during the summer (3.07 per cent), and the highest in the autumn (3.79 per cent); while birth statistics for the city of Philadelphia covering a period of ten years (Sozinskey, '85) and also those for Boston during 1891 to 1910 (Whipple, '19) indicate no appreciable variation in the rate of stillbirths during different seasons of the year.

From available evidence it would appear that the birth mortality in man is but little influenced by the season of the year in which either conception or birth occurs.   Since man has a highly developed mechanism for heat regulation, moderate changes of temperature have very little effect on body metabolism and therefore cannot, under ordinary circumstances, influence the nutrition of the fetus, as Düsing claims.

POSTNATAL MORTALITY

Since deaths that occur among the young within a few days after birth are traceable, in many cases, to prenatal causes that are responsible for a certain proportion of stillbirths, a brief consideration of postnatal mortality is included in the present paper.

Little exact information is available, as yet, regarding the mortality among young rats during the week after birth.   The number of such deaths in any large colony is considerable, but what proportion of them is due to prenatal causes cannot be determined, since a great part of such mortality is always due to causes that are purely accidental, such as smothering of the young by the crowding of adults into the nest when they are cold or

frightened and death from exposure or starvation when the young leave the nest and are not carried back by the mother.

Records kept from June 1, 1918, to June 1, 1919, show that ninety-eight rats died within three days after birth from causes that were undetermined. As during this year 4,250 living young were born in the colony, the postnatal mortality was 2.3 per cent, or slightly greater than the birth mortality during the same period (table 2), Although the great majority of these deaths were undoubtedly accidental, some of them were unquestionably due to prenatal causes that so affected the constitutional vigor of the individual that death was inevitable. Occasionally litters are cast in which one or more of the members are very much under normal size. These small individuals are the so-called 'runts,' which are frequently found among multiparous mammals, and since they are usually unable to compete with the larger and more vigorous individuals of the litter in their efforts to obtain food, they generally die within a few days after birth. Under very favorable conditions some of these undersized individuals are able to survive and to reach maturity, but they never attain the size of the normal members of the litter and they are usually sterile (King, '16). Since runts are found most frequently in very large litters cast by young females and in litters cast by females that are not in good physical condition, they are evidently individuals with relatively low initial vitality that were subjected to conditions inimical to growth during the intrauterine period; the weaker among them die soon after birth, those that survive are among the physically unfit that generally 'drop out' at a relatively early age.

Among the 98 young recorded as dying shortly after birth there were 42 males and 56 females, or a sex ratio of 75 males to 100 females as contrasted with a sex ratio of 141 males to 100 females in the stillborn young found during the same period (table 1). The marked difference between these two ratios is readily explicable. Factors responsible for the great excess of males among the stillborn can act only to a very limited extent in influencing the sex ratio in the young that die after birth, and postnatal mortality due chiefly to accidental causes might be ex-

pected to take a heavier toll from the females, since at birth the females are somewhat smaller, as a rule, than the males (Donaldson, '06; Jackson, '13; King, '15).

The question of postnatal mortality among human offspring involves so many different factors that an adequate consideration of the subject cannot be attempted here. The many investigations that have been made show that the mortality is very high during the first month after birth, averaging about 5 per cent of all young. About one-fourth of these deaths, it has been estimated, are due to improper care or to disease, the remaining can be attributed to premature birth, injuries at birth, or to congenital debility (Ashby, '15; Hunter, '18; Eastman, '19; Dempsey, '19).

Sex statistics for infants dying under one year of age, as collected by a number of investigators in various countries (Düsing, '84; Rauber, '00; Prinzing, '06; Nichols, '07; Dutton, '10; Pinard et Magnan, '13; Kroon, '17; Ashby, '15; Davis, '18, '19, etc.), all show that infant mortality is considerably greater among boys than among girls, and that, while it varies considerably in different localities and under different conditions, on the average about 120 boys die to each 100 girls.

A comparison of the findings for the rat with those for man shows that in both forms the postnatal mortality is somewhat higher than the birth mortality; in the rat this mortality is chiefly due to accidental causes that seemingly tend to kill more females than males, while in man infant mortality is traceable in many cases to 'congenital debility' which is apparently far more fatal to males than to females.

CAUSES OF BIRTH MORTALITY

Barring accidents, there are six leading causes to one or another of which practically all stillbirths in mammals can be ascribed: 1) malposition of the fetus leading to abnormal development; 2) infectious disease; 3) mechanical obstruction to birth, including size of the fetus; 4) physical condition of the mother; 5) age of the mother; 6) congenital debility. The part played by these various factors in the birth mortality in the rat and in man will be discussed briefly in the following sections.

a. Malposition of the fetus and disease as causes of birth mortality

Faulty implantation is responsible for the abnormal development of many ova in the rat (Huber, '15), but these ova, as a rule, die at an early stage and are absorbed in situ. Little is known, as yet, regarding the death in utero of older embryos. The examination of a number of gravid females indicates that this phenomenon is not as common in the rat as it is in many other multiparous mammals (Stahl and Henneberg, '02; Hammond, '14). So-called 'monsters,' which arise through faulty implantation and consequent inadequate nutrition of the embryo, comprise about 1 per cent of all human fetuses at birth (Mall, '08), but they are very rare among newborn rats. In the course of an examination of over 50,000 young rats I have found but four such fetuses, and in all of these the body appeared perfectly normal, but the head was hydrocephalic.

Infectious diseases are responsible for an appalling number of deaths among human offspring and among the young of cattle, but no cases are known, as yet, in which stillbirths in the rat could be ascribed to this cause. Neither the rat scourge, so-called 'pneumonia,' nor other diseases common to the rat are transmitted to the fetus as far as is known. From the evidence at hand, therefore, infectious disease can be eliminated as a cause of stillbirth in the rat, though illness of the mother, as will be shown later, is a potent factor in birth mortality.

b. The size of the fetus as a cause of birth mortality

Since the size of the fetus is an important factor in human birth mortality (Düsing '84; Nichols, '07; Dutton, '10; Hirsch, '13), it is conceivable that this factor may also play a rôle in the birth mortality in the rat. The following series of observations was made to determine this point.

Fifty-nine litters of rats, in which one or more members were stillborn, were obtained at birth. Each of the young rats was taken from the mother as soon as it was cast, the placenta was removed, and the body weight taken immediately. Data regarding the age and general physical condition of the mother at

the time of parturition were also recorded, since these factors had to be taken into account as possible causes of birth mortality.

The body weight records for the 306 living and for the 137 stillborn young in these fifty-nine litters are shown in table 5. For purposes of later analysis the data are arranged in three groups according to the physical condition of the mother at the time of parturition.

TABLE 5

Sex and body-weight data for living and for stillborn young in fifty-nine litters of albino rats. Groups arranged according to the physical condition of the mother at the time of parturition

GROUP	PHYSICAL CONDITION OF MOTHER	LIVING YOUNG						STILLBORN YOUNG						Percentage stillborn in total number of young
		Number of individuals		Average body weight all individuals		Average body weight largest individuals		Number of individuals		Average body weight all individuals		Average body weight largest individuals		
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
				gms.	gms.	gms.	gms.			gms.	gms.	gms.	gms.	
1	Good	68	51	4.57	4.28	4.91	4.52	21	21	4.20	3.75	4.50	4.11	26.1
2	Poor	43	60	4.24	4.17	4.45	4.42	46	28	4.15	4.08	4.40	4.31	41.8
3	Good, but female young	43	41	4.12	3.84	4.53	4.40	12	9	3.56	3.80	3.85	3.85	20.0
		154	152	4.41	4.13	4.66	4.39	79	58	4.11	3.81	4.36	3.99	30.9

In table 5 the final averages, computed from individual not from grouped data, confirm the findings of Donaldson ('06), of Jackson ('13), and of King ('15), that in the rat the living male is heavier at birth than the living female. They show, also, that this same relation is found among the stillborn; the difference between the body weights of the two sexes averaging about 0.30 gram in each case.

In each of the three groups given in table 5, the living young, both males and females, have a heavier birth weight than the stillborn young, the final averages indicating a difference of 0.30 gram for each sex. Obviously the body-weight relations between the living and the stillborn young would be just the reverse of that shown above if the size of the individual is a determining cause of birth mortality. When the average body weights for

the largest of the living and the largest of the stillborn in each group are compared, the result shows conclusively that the size of the fetus is not a cause of death at birth, since in all three groups the average body weight of the largest living individual of both sexes exceeds that of the largest stillborn individual in the corresponding group. In this instance, also, the final averages show a difference of 0.30 gram in favor of the living young.

In man multiple births are the exception, not the rule as in the rat, so in this respect conditions in these two species are radically different. It is not so much the weight of the fetus as the size of the head that is responsible for the death of many infants, particularly boys. The deaths from this cause and those due to other forms of mechanical obstruction to birth comprise about 10 per cent of all human stillbirths, according to various observers.

The data given in table 5 show that the stillbirths formed 30.9 per cent of the total of 443 births in fifty-nine litters of albino rats. In collecting these data there was no error, since the litters were obtained at the time of birth and all of the young recorded. In the total of 253 litters containing stillborn young that were obtained during a period of five years, the birth mortality among 1817 individuals, as registered, was 22.8 per cent (table 1). The difference between these two sets of data would seem to indicate that at most 8 per cent of the stillbirths in the colony were not recorded. This error is not sufficiently large to invalidate any of the conclusions drawn from the records as they stand.

c. The physical condition of the mother as a cause of birth mortality

It requires but little experience in the handling of albino rats to determine from the general appearance of an animal whether or not it is in good physical condition. Alert animals of large size, having clear eyes and thick, glossy hair, are usually in excellent condition and free from disease. On the other hand, labored breathing, rough hair, dark red eyes, sluggish movements, and relatively light body weight are all evidences of poor physical condition and generally indicate that the animal is in an advanced stage of 'pneumonia.'

Table 6 shows the age, body weight, and general physical condition of the fifty-nine albino females that gave birth to the young whose body-weight data are given in table 5.

The first group in table 6 comprises twenty females that were apparently in good physical condition at the time of parturition, as was indicated not only by their general appearance and behavior, but also by the fact that they weighed, on the average, over 15 grams more than the 'standard' body weight for breeding females of the same age (Donaldson, '15). The average body weights of their young at birth exceeded those of the young cast by females in poor condition (cf. group 1 and group 2; table 5), thus adding more evidence that "rats in good physical

TABLE 6

Data regarding the age, body weight, and general physical condition of the fifty-nine female albino rats that cast the litters recorded in table 5

GROUP	GENERAL PHYSICAL CONDITION OF FEMALES	NUMBER OF FEMALES	AVERAGE AGE OF FEMALES AT PARTURITION	AVERAGE BODY WEIGHT OF FEMALES AT PARTURITION	STANDARD BODY WEIGHT FOR AVERAGE AGE OF FEMALES AT PARTURITION (DONALDSON, '15)
			days	*gms.*	*gms.*
1	Good	20	202	224.4	209.1
2	Poor	26	244	190.7	220.0
3	Good; but female very young	13	99	143.7	146.2

condition bear young with a birth weight considerably above that of the young cast by females in poor condition" (King, '15). Each female in this group gave birth to a litter that contained, on the average, two stillborn to six living young (table 5). Since these stillbirths cannot be ascribed either to mechanical obstruction to birth, to abnormal development, nor to infectious disease, it would appear that they must have been caused by some other prenatal condition that adversely affected the vitality of the young.

Examining the history of the mothers, as kept on record cards, it was found that five of the females in this group were nursing young at the time that the litter containing stillborn young was

cast; eight of the females gave birth to very large litters containing ten or more members; three females were over fifteen months of age at the time of parturition.   No reason can be assigned for the presence of stillborn young among the offspring of the remaining four females in this group.   It is possible, perhaps, that these females were in early stages of 'pneumonia' which had not as yet altered either their general appearance or their body weight, but had already affected their body metabolism in such a way as to adversely influence the development of the fetal young.

The second group in table 6 comprises twenty-six females that were obviously in bad physical condition at the time that their litters were cast.   These females were, on the average, some 30 grams under the 'standard' body weight for breeding females of the same age, and the birth weights of their young were very low (table 5).   It is of interest to note that the percentage of stillbirths in the total number of young cast by these females was relatively very high (cf. group 1 and group 2; table 5).   The majority of the females in this group were obviously suffering from 'pneumonia;' three of them were in such an advanced stage of the disease that they had to be killed as soon as the litter was cast.

d. The age of the mother as a factor in birth mortality

It has already been shown that litter size in the albino rat is influenced to a considerable extent by the age of the mother (Slonaker, '12; King, '16 a), and it is possible that the viability of the young at birth may also be affected by this same factor.

As already noted, three of the females in the first group of table 6 were over fifteen months of age when casting a litter that contained stillborn young.   These females appeared to be in good physical condition, yet their litters were very small, and seven of the eleven young cast were dead at birth.   The third group of table 6 comprised thirteen females that had an average age of only ninety-nine days when casting their first litter.   Although each of these females was seemingly in good health, twenty-one in the total of 105 young were stillborn (table 5).

Since none of the females in these two groups showed any evidence of disease at the time of parturition, it is probable that the age of the mother, and not incipient 'pneumonia,' was the chief factor responsible for the high birth mortality in their young. The age of eighteen months marks approximately the end of the reproductive activity of the albino female, and toward its close, as at its beginning, there seems to be a strong tendency for the females to cast fewer individuals in a litter and a relatively greater proportion of stillborn young. This phase of the subject will be discussed later.

In considering the causes responsible for stillbirths among human offspring, the age of the mother is a factor that is usually ignored or assigned a very minor rôle. Various series of reliable statistics, however, indicate that the birth mortality is relatively high in children of very young and of very old mothers, so evidently the age factor has greater influence in this respect than is generally assumed. The trend of statistical evidence on this point is shown by the following examples. Whipple's ('19) analysis of the birth statistics for the city of Boston during the period from 1891 to 1910 shows that: "The percentage of stillbirths arranged according to the age of the mother gave the very high percentage of 11.1 per cent for mothers under 20 years of age, 4 per cent for age group 20–24, 5.1 for 25–29 years, 4.4 for 30–39 years, and 3.3 for ages over 40."

Far more comprehensive data regarding the effect of the age of the mother on birth mortality among the young are given by Davis ('20) in his study of the births in the registration area of the United States during the year 1918. In a total of 1,372,329 births in which the age of the mother was ascertained, there were 46,122 stillbirths. The percentage of stillbirths was 3.9 for mothers under twenty years of age, 3.2 per cent for mothers from twenty to thirty-nine years, and 5.4 per cent for mothers over forty. This study indicates that the percentage of stillbirths is much higher in children born to mothers at the beginning and at the end of the reproductive period, and its findings are confirmed by the birth statistics gathered by The Children's Bureau (Duke, '15; Duncan and Duke, '17; Allen, '19; Dempsey, '19),

which have already been given in table 3 of the present paper. They are shown again in table 7, arranged according to the age of the mother at the time that the birth of her child occurred. It will be noted that table 3 gives a total of 5,456 births, while table 7 registers only 5,452 births. This discrepancy is due to the fact that in four cases the age of the mother was not ascertained.

Nearly 90 per cent of the births registered in table 7 were those to women between twenty and thirty-nine years of age, the remaining 10 per cent were evenly divided between women that were under twenty and over forty. As this is about the normal distribution of births relative to the age of the mothers, a comparison of the percentages of stillbirths as given seems permissible.

TABLE 7

Data for human births, collected by the U. S. Children's Bureau (table 3), arranged according to the age of the mother at the time of parturition

MOTHER'S AGE IN YEARS	TOTAL NUMBER OF BIRTHS	LIVING BIRTHS	STILLBIRTHS	PER CENT STILLBIRTHS IN TOTAL NUMBER OF BIRTHS
Under 20	282	263	19	6.95
20 to 39	4,882	4,683	199	4.07
40 and over	288	268	20	6.94
	5,452	5,214	238	4.36

Table 7 shows clearly that in this set of records, as in those given by Whipple and by Davis, the percentage of stillbirths is correlated with the age of the mother. In table 7 the stillbirths formed only 4.07 per cent of all births to women at the zenith of the child-bearing period, while they were increased to nearly 7 per cent in the births to women at the extremes of the reproductive period.

When the data in table 7 are arranged according to the order of the birth, as is shown in each of the papers in which the separate sets of data are given, it is found that the percentage of stillbirths is higher for the first births and for those after the sixth than for the intermediate births. A series of birth statistics arranged in this manner is, of necessity, an age series, and it is

more probable that the observed variations in the percentage of stillbirths depend on the age factor rather than on the number of the pregnancy.

It has been claimed that the high birth mortality in children of very young mothers is due chiefly to the mother's ignorance of the proper hygienic laws that should be observed by pregnant women. This explanation cannot be offered to account for the increase in the percentage of stillbirths among the children of women over forty, however, since births at this age are rarely those of the first pregnancy. It seems probable that both at the beginning and at the end of the reproductive period physiological conditions incident to age are responsible in great part for the high birth mortality among the children born at this time.

e. Congenital debility as a cause of birth mortality

Stillbirths among human offspring not traceable to a well-defined cause are generally ascribed to 'congenital debility,' this term being used to indicate a lack of sufficient vitality in the fetus to render postnatal existence possible. Various series of investigations show that a very considerable proportion of stillbirths are attributed to this cause. For example, in 201 cases of stillbirths at term studied by Brothers ('96), over 50 per cent were classed as due to 'congenital debility,' while Waldvogel's ('13) studies led to a similar conclusion.

In the sense in which the term 'congenital debility' is used above, practically all stillbirths in the rat might properly be grouped under this heading, since in all cases so far found impaired vitality of the fetal young was seemingly the direct cause of the birth mortality; the underlying cause is discussed in the following section.

GENERAL DISCUSSION

This study has shown that in two important respects the statistics for the birth mortality in the albino rat accord in a most striking manner with those for man: 1) the sex ratio in the living young at birth is practically the same in both forms (about 105.5

males to 100 females); 2) the great excess of males among human
stillborn finds its parallel in the high sex ratio which charac-
terizes the stillborn of the rat (129 males to 100 females).   In
one respect only the birth records for these two forms do not
agree.   The normal percentage of stillbirths in human offspring
(4 to 5 per cent) is at least twice that in the albino rat (table 2).
This difference, however, is readily explicable.   Factors which
are responsible for about one-half of all human stillbirths, i.e.,
mechanical obstruction to birth, accidents, faulty implantation,
and infectious disease, ordinarily play little, if any, part in the
birth mortality in the rat.   If stillbirths due to these causes are
eliminated, the birth mortality among human offspring falls to
about 2 per cent, which is close to the percentage of stillbirths·
which is seemingly normal for the albino rat when large numbers
of breeding animals are kept under fairly uniform conditions of
environment and of nutrition.   The stillbirths in man which
thus seem comparable to those in the rat, are those that, in gen-
eral, are attributed to 'congenital debility,' this term, as already
stated, being used to indicate that the fetus possesses such a
low state of vitality at the end of term that it is incapable of inde-
pendent existence.   From the evidence at hand the great pro-
portion of stillbirths in the rat can be attributed to the same
cause.

The question at once arises as to the cause of this impaired
vitality in the fetus and whether it is possible to control it so
that the percentage of stillbirths will be materially decreased.
If we consider those cases in the albino rat in which the stillborn
young were obtained at the time of birth and the age and physical
condition of the mothers noted (tables 5 and 6), it is found that
the great majority of them occurred in litters of females that were
suffering from disease, chiefly 'pneumonia,' or in those of females
at the extremes of the reproductive period, while in a few cases
the litters were very large or were cast by females that were
suckling young at the time of parturition.   Extensive series of
breeding experiments extending over a period of a dozen years
and covering the birth of many thousands of rats lead me to
believe that practically all stillbirths in this animal occur under

one or another of these conditions. The one factor which seemingly might have affected the vitality of the fetal young in all of these cases is malnutrition.

The nutrition of the fetal young is a very complex process, and in its broadest sense it includes the absorption and the assimilation of food by the mother as well as its transmission through the placenta to the young. Any factor or physiological condition that adversely affects the normal metabolic processes upon which any phase of embryonic nutrition depends therefore indirectly influences the development of the young and may impair their vitality to a greater or less extent.

Let us consider in some detail the effect of the various factors that are apparently responsible for stillbirths in the rat. The rat scourge, 'pneumonia,' is a wasting disease, and as such profoundly affects all of the normal life processes in an animal affected with it. The living young cast by females having this disease in an advanced stage are usually very small and emaciated at birth, and their vitality is at such a low state that they are difficult to rear even when suckled by a vigorous foster-mother. There can be no question but that the fetal young suffer throughout the entire course of their intra-uterine existence from malnutrition, since the illness of the mother must interfere with her power to assimilate food and to transmit it to her offspring. With such a handicap to their normal development, it is not surprising that a large proportion of the young are not able to survive at birth.

The suckling of young, particularly if the litter is above the norm (seven) in size, is as a rule a severe strain on the nutritive reserve of the mother, as is shown by the fact that she usually loses considerable weight during this period unless she is in excellent health and abundantly supplied with food. Not infrequently a lactating female is also carrying a second litter. If one or both of these litters are large, the amount of food that the mother can assimilate and supply to her young, in addition to her own needs, is inadequate for the proper nutrition of all of the individuals concerned. The result is that the suckling young usually grow very slowly and show every evidence of being un-

derfed, and the gestation period of the fetal young is lengthened from one to several days, owing doubtless to the fact that the implantation of the fertilized ova is delayed (King, '13; Kirkham, '16). Here again malnutrition is obviously a factor that impairs the vitality of the fetal young and tends to increase the proportion of stillbirths. A similar explanation can be offered for the presence of stillborn young in litters of exceptionally large size. In these cases the inadequate nutrition of the young is indicated by the fact that all members of the litter are, as a rule, of small size and under normal weight at birth.

The fact that the proportion of stillborn young in litters cast by very young and by very old females is markedly greater than that in litters cast by females at the height of their reproductive activity indicates that the age of the mother is a factor of importance in birth mortality. Donaldson ('06) has shown that one year of a rat's life is equivalent to thirty years of human life. At the age of eighteen months, therefore, a female rat corresponds physiologically to a woman of forty-five years, and it can hardly be a coincidence that this age marks approximately the end of the reproductive activity in both species. The onset of puberty does not, however, correspond as closely in the two forms, since rats breed at three months of age. Age has a profound effect on all of the normal activities of the body, and it is not surprising, therefore, to find that the immaturity of the young mother and the physiological changes in the uterus incident to the approaching menopause seemingly inhibit the metabolic processes concerned with the nutrition of the fetal young. In such cases the young often suffer from impaired nutrition, and consequently the birth mortality among them is much greater than that among the offspring of females at the height of their reproductive activity.

It has been repeatedly demonstrated that a mature, well-developed albino female that is in good physical condition at the time of conception, will, if abundantly supplied with proper food during the entire gestation period, cast a litter containing only living, vigorous young that have relatively heavy birth weights. A special experiment recently made to test this point has given

rather astonishing results. The young cast by a mature female abundantly supplied with rich food during the gestation and lactating period were twice the normal weight at birth, and at thirty days of age, when weaned, they were over 200 per cent above the average weight of rat at this age. At sixty days of age the males in this litter had an average body weight of 260 grams, which is some 300 per cent above the 'standard' weight for males of this age. On the other hand, inadequate feeding of breeding females invariably leads to the production of small litters containing undersized individuals in which there is a high percentage of stillbirths. This fact was fully demonstrated in the early stages of an inbreeding experiment with these animals which has been carried on for some years in our colony (King, '18). Even when the food supply is ample, the present study has shown that in lactating females, in those carrying a very large litter, and in those suffering from disease or breeding at one extreme of the reproductive period, physiological conditions within the body of the mother may adversely influence the various processes upon which the nutrition of the young depends and thus lead in many cases to the death of a considerable proportion of the young at birth.

Conditions of human existence are so complex and so artificial in many cases that one would hardly venture to assert that the underlying cause of all cases of 'congenital debility' was malnutrition of the fetus due to the age or to some abnormal physical condition of the mother, yet the evidence at hand is strongly in favor of such a view. Inadequate nutrition of the young is certainly a potent factor in the birth mortality of the rat, and it probably plays an important rôle in the birth mortality of other mammals, including man. The maintenance of pregnant females under environmental and nutritive conditions favorable to the health of the mother and to the adequate nourishment of the fetal young throughout the entire gestation period, not merely near its close, would, therefore, undoubtedly lead to the birth of more vigorous young and to a marked decrease in the number of abortions and of stillbirths.

Whenever large groups of human birth statistics have been analyzed it has been found that the mortality among the males is very high, the sex ratio for the stillborn being at least 25 points higher than that in the living young.   This same phenomenon appears also when a large series of birth data for the rat are examined (table 1), and it likewise is present in statistics for the horse, if Goehlert's ('82) records are reliable.   Since the disparity between the sex ratios for the stillborn and those for the living young in these various groups of statistics is fairly constant and much too great to be considered as within the range of normal variation in the sex ratio, there must be some fundamental cause, founded on a difference in the constitution of the male and female organisms, that is responsible for the excessive mortality among the males at birth.

Düsing ('84) discusses this subject at considerable length, and he concludes that: "Die Knaben sterben also während Fötallebens häufiger als die Mädchen, weil viele derselben sich unter ungünstigen Ernährungsverhältnissen ausbilden, während sie, da sie durchschnittlich schwerer sind, sogar mehr Nahrung beanspruchen als die leichteren Mädchen." Since from the time that the sexes can be distinguished at about the sixth week of pregnancy the excess of males among aborted embryos is greater the earlier the age at which abortion occurs (Rauber, '00; Nichols '07; Auerbach, '12; Carvallo, '12), inadequate nutrition cannot well be considered as the primary cause of the greater mortality among male fetuses in general.

A most suggestive hypothesis that may have a very important bearing on this problem has recently been advanced by Lillie ('17) in his study of the action of sex hormones in producing the 'free-martin' in cattle: "It seems probable that the disturbance of the equilibrium that protects the male from the sex hormones of the mother would result in malformations of the male sex characters to a degree commensurate with the extent of the disturbance.   There is, therefore, here a possible explanation for the greater mortality among male foetuses."   Our knowledge of the action of sex hormones is as yet too meager to enable us to

advance a definite theory regarding the influence of these factors on fetal mortality.

Nichols ('07) states that three explanations are open to consideration to account for the heavy mortality among boys at birth "(a) the initial numerical preponderance of males; (b) the greater proportion of deaths of male fetuses occurring during parturition owing to their larger size; and (c) the intrinsically much greater mortality of males than of females in the earlier period of life, both antenatal and postnatal." Nichols assigns only a minor rôle to the first two of the causes enumerated above, and he concludes that:

Obviously the main cause of the great preponderance of male stillbirths resolves itself into the question of the comparative mortality or death rate of the male and female sexes during the intrauterine period of existence. . . . . it is therefore obvious that the male constitution is intrinsically weaker, less hardy, and more susceptible to morbific and mortific influences, and has less vitality and resisting power against disease, than the female. The cause of this innate disparity of vitality between the two sexes we do not know; but the fact that it exists, that the antenatal mortality and death rate of males much exceeds that of female fetuses, accounts for the great excess of male over female stillbirths.

In the light of the recent researches in heredity it is conceivable that the inherent dissimilarity between the sexes as regards their constitutional vigor, which has been discussed in detail by Geddes and Thomson ('01), may have its basis in the germinal structure of the fertilized egg. From the work of Guyer (10) and of von Winiwarter ('12) on the spermatogenesis of man, and of Allen ('18) on the spermatogenesis of the rat, it is known that in both of these mammals the spermatozoa are dimorphic, one kind of spermatozoa having one more chromosome than the other; both kinds of spermatozoa are produced in equal numbers and both kinds, as far as known, are equally functional. The current theory of sex determination postulates that the spermatozoa containing the extra, or X-chromosome, are 'female-producing'; those lacking it are 'male-producing.' The fertilized ovum that is to develop into a female thus contains two X-chromosomes, while that having only the X-chromosome received from the mother

develops into a male.   May not the difference in the constitutional vigor of the two sexes depend, in some way, upon the fact that the chromatin content of the female ovum is greater than than of the male ovum?   One might suggest, perhaps, that the excess of chromatin brought into the egg by the 'female-producing' spermatozoan influences the ensuing interaction of the chromatin and the cytoplasm in such a way that the embryo becomes endowed with a constitution that is more stable and more vigorous than that of the embryo developing from an egg in which the initial amount of chromatin is less.   Such an hypothesis is, of course, only tentative.   Until our knowledge of heredity and of the sex-determining mechanism is greatly increased it will be futile to theorize as to the probable cause for particular characters associated with one sex or the other which seemingly do not depend upon the action of specific genes.

The great excess of males among aborted and stillborn fetuses is readily explained if we assume that, from the time of conception, the embryo that is to develop into a male has a constitution inherently weaker than that of the embryo that is to become a female.   During the gestation period many factors, such as disease, unfavorable environmental conditions, physiological changes incident to age, etc., may lessen the mother's power of assimilating food and of transmitting it to her fetal young.   Under these conditions, a male fetus possessing a relatively low initial vitality would be more severely handicapped by inadequate nutrition or by conditions unfavorable to normal development than would a female fetus having a greater initial vigor of constitution and more power to resist unfavorable environmental conditions. One, therefore, would expect to find the facts exactly as shown by various series of investigations cited in this paper, namely, that at all stages of gestation and at birth the mortality among the males is far greater than that among the females.

SUMMARY

1. Birth statistics collected during a period of five years show that in a total of 31,670 newborn albino rats 415, or 1.3 per cent, were stillborn.   Allowing for the probable error in recording the

data, it would appear that under normal environmental conditions not more than 2 per cent of rat fetuses are dead at birth.

The most accurate statistics available indicate that the normal birth mortality in man is about 4 per cent. There are no data of value regarding the percentage of stillbirths in other mammals.

2. The normal sex ratio in newborn albino rats, including the stillborn, is about 107 males to 100 females; in man the sex ratio for the living young at birth averages about 105.5 males to 100 females, and if the stillborn are added the sex ratio rises to about 108 males to 100 females.

3. In each year that the mortality records for newborn rats were taken there was a pronounced excess of males among the stillborn, the sex ratio in the total number of such individuals being 129.3 males to 100 females (table 1).

Large series of birth statistics collected in many different countries show that the sex ratio among stillborn children is very high, averaging about 130 to 140 boys to 100 girls. The excess of boys becomes greater the earlier the month of pregnancy in which the fetus dies.

4. In the rat the percentage of stillbirths seems to vary somewhat with the seasons, being least in the spring and greatest in the autumn months when the breeding animals are suffering from the devitalizing effects of high temperature during the preceding summer (table 4). The birth mortality among human offspring does not, apparently, vary to any appreciable extent at different periods of the year.

5. Data collected during one year show that the mortality among young rats during the first three days after birth was 2.3 per cent, or slightly greater than the birth mortality in the colony during the same period (2.16 per cent); infant mortality in man during the first month after birth is about 5 per cent, or 1 per cent higher than the birth mortality.

Postnatal mortality in the rat is largely due to accidental causes which tend to kill more females than males; in man the death of many infants is traceable to prenatal causes which seemingly are more fatal to boys than to girls.

6. Factors responsible for a considerable proportion of the stillbirths among human offspring, such as, infectious disease, faulty implantation, mechanical obstructions to birth, including the size of the fetus, apparently play no part, ordinarily, in the birth mortality of the rat.

7. From the data obtained it appears that malnutrition is directly responsible for most of the stillbirths in the rat. Factors influencing the food supply of the fetal young, such as the physical condition and the age of the mother, the suckling of young, and the size of the litter carried, are therefore the chief causes of birth mortality in the rat.

8. Available evidence indicates that both in the rat and in man the male fetus is intrinsically weaker than the female, and therefore more susceptible to prenatal influences inimical to normal development. A tentative hypothesis is advanced that the difference in the constitutional vigor of the sexes has its basis in the different chromatin structure of the male and female zygote.

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By HELEN DEAN KING

From the Wistar Institute of Anatomy and Biology

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From The Wistar Institute of Anatomy and Biology

SIX FIGURES

A study of the mature spermatocytes in the testes of Necturus maculosus has shown that these cells contain a peculiar multiple chromosome that divides unequally in the first maturation mitosis and thus leads to the formation of two classes of spermatozoa, one having more chromatin than the other. As a dimorphism in the spermatozoa of amphibians has not heretofore been reported, a brief description of the spermatocyte divisions in Necturus is given in the present paper: a more detailed account will appear when my investigation of the early growth stages of the spermatocytes has been completed.

In a very early prophase of division the primary spermatocytes contain a thick, apparently continuous spireme which is split longitudinally throughout its entire length. This spireme breaks into twelve segments of different lengths which condense rapidly to form the chromosomes for the first maturation spindle.

Two of the chromatin segments of the prophase are distinguished from the others on account of their greater length. One of these segments becomes a very large ring-shaped chromosome which is very conspicuous in the metaphase of the first mitosis when it is usually twisted in various ways (figs. 2, *L*; 3, *L*). In mitosis this chromosome divides equally, forming two *V*-shaped chromosomes which move to opposite poles of the spindle. The other long segment, as its later history shows, is not a bivalent but a multiple chromosome. In the late prophase this segment appears as a long, thick rod of nearly uniform diameter with the

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two ends bent at nearly right angles to the main axis (fig. 1). The bent terminal sections are never of equal length, and the longer one is the 'accessory' or the 'X chromosome' which became attached to one of the large bivalent chromosomes at an early period in the growth of the spermatocytes. Not infrequently, as shown in figure 1, X, the X chromosomes is detached from the main structure and connected with it only by linin.

The multiple chromosome goes into the first maturation spindle in practically the same form in which it appears in the late prophase, and in the metaphase it assumes such a position on the spindle that its main axis lies along one of the spindle fibers while the two bent terminal portions either project at right angles to the spindle (fig. 2), or they lie across the spindle fibers (fig. 3). There is more or less variation in the size of this chromosome in different spindles, due doubtless to fixation and staining, but in nearly all cases it extends over considerably more than half of the length of the spindle and in some cells it reaches from pole to pole. Sometimes this structure is of nearly uniform thickness throughout its entire length (fig. 3): more frequently, as shown in figure 2, it has slight constrictions that divide it into five parts and thus give it the appearance of a pentad structure. In its constricted form this chromosome appears remarkably like the multiple chromosome found by McClung ('05) in the spermatocytes of Hesperotettix speciosus and of various other species of orthoptera.

Whenever the multiple chromosome is shown in its entirety the portion of it which forms the X chromosome can readily be distinguished. Sometimes the X component seems to be an integral part of the multiple chromosome, although it invariably forms a sharp angle with the main axis of this structure (fig. 2, X). At other times, as shown in figure 3, the X chromosome is a short, rod shaped body lying close against one end of a large bivalent chromosome, but apparently not connected with it in any way.

The bent portion of the multiple chromosome at the end opposite to the X chromosome is usually much shorter than the X component (figs. 2 and 3), and as yet I have not found a single case in which this section was not directly continuous with the main axis of the multiple chromosome although always forming an angle

with it.   It seems probable, from the evidence in hand at present, that this bent terminal portion is merely the end of one of the

All figures were drawn with the aid of a camera lucida under a magnification of about 1500 diameters: they have been reduced one-half.   *L*, large ring-shaped chromosome; *S*, supernumerary chromosomes; *X*, *X* chromosome.
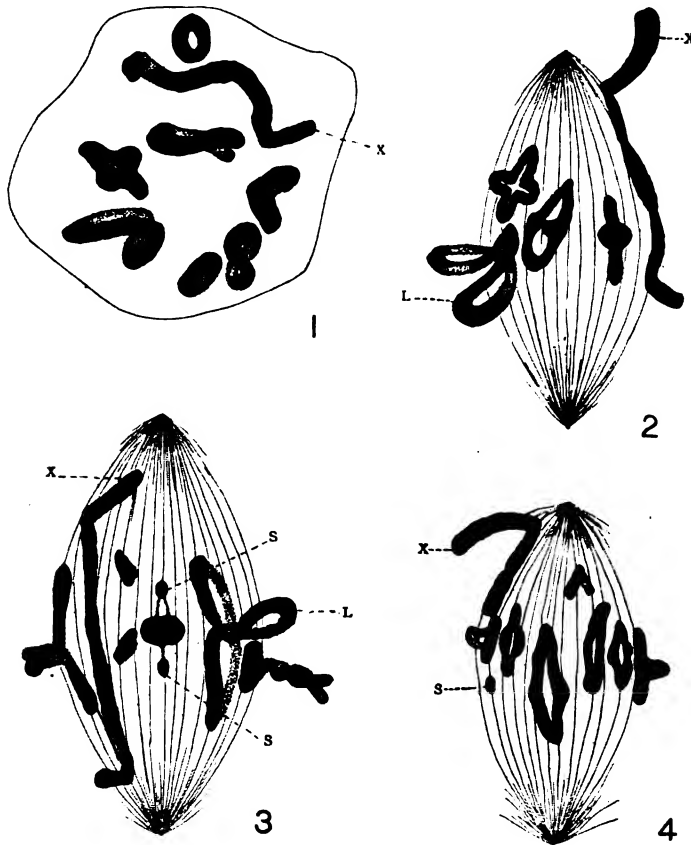
Fig. 1   Late prophase of the first maturation mitosis.
Figs. 2-4   Metaphases of the first maturation mitosis.

univalent chromosomes which helped to form the multiple chromosome during an early period in the development of the spermatocytes.   There is the possibility, however, that this section is the small mate of the *X* chromosome, in which case the spermato-

cytes of Necturus contain an unequal pair of heterochromosomes instead of an X chromosome. This point cannot be cleared up until the early history of the spermatocytes has been studied.

When the first maturation mitosis occurs the multiple chromosome divides in such a way that one daughter cell gets one univalent chromosome and the X component (fig. 4) while the rest of the complex goes into the other cell.

The other ten segments of the prophase are considerably shorter than the two described above. At least six of these segments appear as small rings in the late prophase (fig. 1) and go into the spindle in this form. All of the remaining segments condense as crosses with one pair of arms considerably longer than the other (figs. 1 and 2). The cross-shaped chromosomes undergo considerable modification in form before the maturation division occurs. At first the longer axis of each of these chromosomes lies along one of the spindle fibers (fig. 2). This axis shortens gradually and its chromatin substance goes into the side arms which increase correspondingly in length. Just before division these chromosomes appear either as short, thick rods which lie across the spindle fibers, or as small elongated rings in which the central opening is a mere slit (fig. 3). In mitosis each of these chromosomes divides through its longitudinal axis, forming two small V-shaped chromosomes of equal size. A similar change in the shape of cross-shaped chromosomes preparatory to division has been found in the germ-cells of various other species of amphibians (Carnoy and Lebrun '99; Lebrun '01; King '05).

In connection with the change in the shape of the cross-shaped chromosomes in the spermatocytes of Necturus there sometimes occurs a process which, as far as I am aware, has not been observed to take place in the germ-cells of other amphibians in which chromosomes of a similar shape are found. A small mass of chromatin, at one or at both ends of the arms lying along the spindle fiber, becomes so firmly attached to the spindle fiber that it gradually becomes separated from the main body as the side arms increase in size. For a time such small fragments remain attached to the rest of the chromosome by linin-like strands (fig. 3), but eventually they break away from the parent mass and appear on the

spindle as small, round supernumerary chromosomes (fig. 4, S). These supernumeraries move with the V-shaped chromosomes to the spindle poles, and they can sometimes be distinguished in a late anaphase. These small chromatin masses are seemingly derived only from the chromosomes that appear on the spindle in the form of a cross, and as they move to the same pole of the spindle as do the univalent chromosomes to which they belong, their detachment from the main mass of chromatin does not lead to an unequal distribution of chromatin to the spindle poles. Supernumerary chromosomes are not found on all spindles and their formation is apparently due solely to the fact that at times the connection between the ends of the cross-shaped chromosomes and the spindle fibers is stronger than the force that is changing the shape of the chromosome and causing the chromatin to move into the side arms. I have not as yet been able to trace these small chromosomes beyond the first maturation spindle. Small supernumerary chromosomes have been found in the spermatocytes of various species of insects by Stevens ('12 a, '12 b) and by Wilson ('09), but they differ from the supernumerary chromosomes of Necturus in that they appear to be derived from the heterochromosomes and not from the ordinary bivalents.

The first maturation mitosis in Necturus is probably a segregation division, and there is seemingly a definite order in which the various chromosomes divide. The multiple chromosome divides after the division of one or of two of the smallest ring-shaped chromosomes (fig. 4); then follows the separation of the univalents which were united as crosses or as rings of medium size; lastly the large ring-shaped chromosome separates into its univalent components. Since the multiple chromosome divides at a very early period it is evident that the order in which the chromosomes divide is not dependent on the size of the chromosomes but on some other factor as yet undetermined.

There is a resting period of considerable length between the two maturation divisions, during which the chromosomes lose their visible identity and form a continuous spireme. The chromosomes that emerge from the spireme to go into the second maturation spindle are V-shaped structures that vary consider-

ably in size. As a rule two of these chromosomes are considerably larger than the others. One, with arms of equal length, is doubtless the univalent chromosome which with its homologue formed the large ring-shaped chromosome of the first maturation spindle. The other large *V*-shaped chromosome has arms that are slightly unequal. This is probably the chromosome derived from the multiple chromosome of the first spindle. Since the chromosomes are very much crowded together in the second spindle it becomes very difficult to follow the *X* chromosome. In most cases the *X* chromosome probably remains attached to one of the univalent chromosomes and divides longitudinally with it
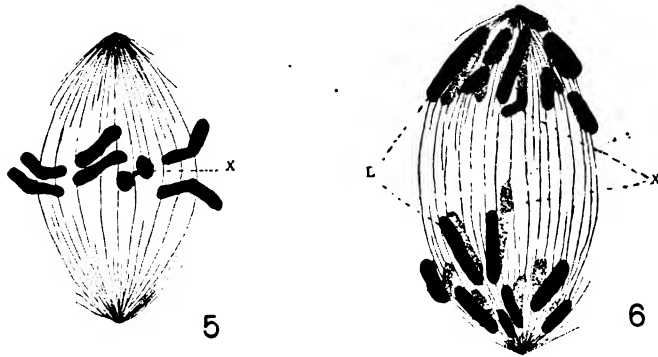
Fig. 5   Early anaphase of the second maturation mitosis.
Fig. 6   Late anaphase of the second maturation mitotis.

(fig. 6, *X*). At times, however, the *X* chromosome apparently breaks away from its attachment to the large chromosome and divides alone in the second mitosis, forming two small, nearly spherical chromatin masses one of which goes to each pole of the spindle (fig. 5).

Since the *X* chromosome passes undivided to one pole of the first maturation spindle and divides longitudinally in the second maturation mitosis two classes of spermatozoa are produced in Necturus, as in many other species of animals. Presumably both classes of spermatozoa are functional, as I have not found sufficient degeneration among the spermatids to warrant the assumption

that spermatocytes lacking the X chromosome degenerate, as is the case in Phylloxerans (Morgan '09) and several other forms.

The mature spermatocytes of Necturus show a condition of the X chromosome which is transitional between that found in the many species in which the X chromosome can be traced as a separate structure throughout the development of the spermatocytes and the chromatin relations in such forms as the higher batrachians in which the X chromosome has apparently formed a permanent union with one of the large chromosomes and can no longer be distinguished by any method of technique at our command. It is hoped that the study of the early stages in the development of the spermatocytes will give further facts of interest regarding the relations of the X chromosome in this amphibian.

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CONTENTS

THE EFFECTS OF HEAT ON THE DEVELOPMENT
OF THE TOAD'S EGG

HELEN DEAN KING

THE EFFECTS OF HEAT ON THE DEVELOPMENT OF THE TOAD'S EGG.

HELEN DEAN KING.

An extended series of experiments made by Hertwig (1–4) prove that the maximum temperature at which the eggs of the frog will develop normally differs for different species. His experiments also show that eggs in the cleavage stages can withstand a higher temperature than can unsegmented eggs. These results have a bearing on the general problem of adaptation ; for it may be possible to show, after more species have been studied, that the maximum temperature which the eggs of amphibians can endure without injury and also the temperature most favorable for their development depend, to a certain extent at least, on the time of year at which the eggs are deposited.

MATERIAL AND METHOD.

The eggs of the common toad, *Bufo lentiginosus*, were used in making all of the experiments recorded in the present paper. After natural fertilization, the eggs were brought into the laboratory where the temperature varied from 18 to 21° C. Control sets of eggs from each lot used for the experiments, developing at the room temperature, all became perfectly normal embryos, and some of them were kept until metamorphosis.

In making the experiments, small dishes containing about 80 c.c. of spring water were placed in the drying chamber of a large water-bath, and after the water had become heated, from 50 to 75 eggs were quickly transferred into it and left a given length of time. The temperature to which the eggs were being subjected could readily be told from a thermometer that projected into the chamber through a small opening in the top. Great care was taken to keep the temperature of the chamber as constant as possible during the course of the experiments, and in no case did it vary more than two degrees. After the eggs were removed from the chamber, they were put into fresh water at room

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temperature and their later development compared with that of the eggs in the control set.

II. EXPERIMENTS ON UNSEGMENTED EGGS.

Experiment 1. — On April 16, twenty-five unsegmented eggs were subjected to a temperature of 28–30° C. for two and one-half hours. When removed from the chamber, all of the eggs were in the 16-cell stage, while in the control set, developing at room temperature, the eggs had only reached the 4–8-cell stage. The immediate effect of the higher temperature, therefore, was to increase the rate of development. This result agrees fully with that obtained by Hertwig in many of his temperature experiments on the frog's egg. The later development of the eggs in this series appeared to be perfectly normal, and it took place at about the same rate as in the eggs of the control set.

Experiment 2. — A number of eggs that had not yet segmented were put into water at a temperature of 30–32° C. on April 17. Part of the eggs were removed at the expiration of three quarters of an hour, and when examined they were all found to be segmenting. In a few cases the first cleavage plane had nearly cut through the yolk portion of the egg and the second furrow was appearing. In the control set of eggs, the first cleavage plane was just coming in at this time, so that, in this experiment also, the early development became more rapid as an immediate result of exposing the eggs to a higher temperature. All of these eggs developed into normal embryos.

Some of the eggs of the above lot remained in the heated chamber for one hour. The second cleavage plane had appeared in all of the eggs when they were removed to room temperature. Later segmentation was normal, and on the following day the dorsal lip of the blastopore appeared in all of the eggs at about the same time that it formed in the eggs of the control set. On April 19, many of the eggs were dead; some were in the early gastrula stages, and some showed traces of the medullary folds. Of the seven embryos alive on April 20, three were abnormal, having a large yolk plug exposed at the posterior end of the body; the other four embryos were normal and were kept for several weeks.

The remaining eggs of this lot were kept at the temperature of 30–32° C. for one and one-half hours.   At the end of this time they were in the 16-cell stage, while the eggs of the control set were only in the 2–4-cell stage.   Later segmentation of these eggs seemed to be normal, and on April 18 the dorsal lip of the blastopore appeared in a very few of them.   On the morning of April 19 most of the eggs were dead, and not one of them, when examined, was found to have gastrulated.   In the eggs still living the blastopore was closing in, but development was much slower than that of the eggs of the control set in which, at this time, the blastopore had already closed and the medullary folds were forming.   All of the eggs were dead on the morning of April 20, and in no case was gastrulation entirely completed.

In these last two lots of eggs the injurious effects of heat were not apparent during the segmentation stages and only manifested themselves when the eggs were ready to gastrulate. Early development was accelerated; but later development lagged behind, or, at most, was equal to that of the eggs in the control set.

Experiment 3. — A number of unsegmented eggs were exposed to a temperature of 32° C. for two hours on April 22, and when removed they were in the 16-cell stage.   In this lot of eggs the later cleavage was very abnormal as the upper hemisphere divided into a number of small cells, while the lower part of the egg segmented only a few times and, consequently, was composed of a small number of very large cells.   Cleavage lines were very distinct in the upper part of the egg; but it was almost impossible to make out the boundaries of the yolk cells.   None of the eggs in this set gastrulated and all of them were dead by April 24.

Experiment 4. — On the morning of April 16, a small lot of eggs was subjected to a temperature of 32–33° C. for one-half of an hour.   The eggs had not segmented when they were put into cooler water, but in every case the first furrow appeared in about fifteen minutes.   In the control set, the first cleavage plane came in about half an hour later than it did in the eggs used for the experiment.   All of the eggs of this set developed

normally, and sections made of later embryos showed them to be no different from the embryos of the control set.

Experiment 5.— A bunch of about seventy-five unsegmented eggs was put into water heated to a temperature of 34–35° C. on April 16. Part of the eggs were removed at the end of half an hour and a few of them at once began to segment. None of the cleavage planes, with the exception of the first, came in normally, and in no case did any of them cut through the entire egg. Part of a section of one of these eggs is shown in Fig. 1. All of the cleavage planes are seen to be parallel and to extend but a short distance through the upper hemisphere of the egg. Development did not progress beyond this stage in any case, and the majority of the eggs never segmented although they appeared to be living several hours after they were brought into room temperature.

Some of the eggs of the above lot remained at the temperature of 34–35° C. for one hour. When put into cooler water and examined, a slight depression was found in the center of the upper hemisphere of a few of the eggs as if the first cleavage plane was about to appear in its normal position. This appearance, however, proved to be only a wrinkling of the surface as none of the eggs, when sectioned, showed any true cleavage planes.

The above experiments show that the unsegmented eggs of the toad can withstand a temperature of 32–33° C. for one-half of an hour and develop normally, while an exposure to this temperature for a longer period is very injurious and only a small per cent. of the eggs produced normal tadpoles. Exposure to a temperature of 34°, even for a short time, injures the eggs beyond the possibility of a recovery. The maximum temperature that the unsegmented egg can endure without injury is, therefore, 33° C. The optimum temperature, a term defined by Hertwig (3) as, "Die Temperatur bei welcher sich der Entwicklungsprocess bei allen Eiren mit der grössten Beschleunigung ohne eine auffällige Störung und Abweichung von der Norm vollzieht," for this egg is probably not far from 28° C., judging from the results obtained in experiments 1 and 2. In all cases in which the heat did not kill the eggs, development was accelerated at first, apparently with no injurious effects on the egg. In later stages,

1

2

S. C.

3

S. C.

4.

S. C.

Bl
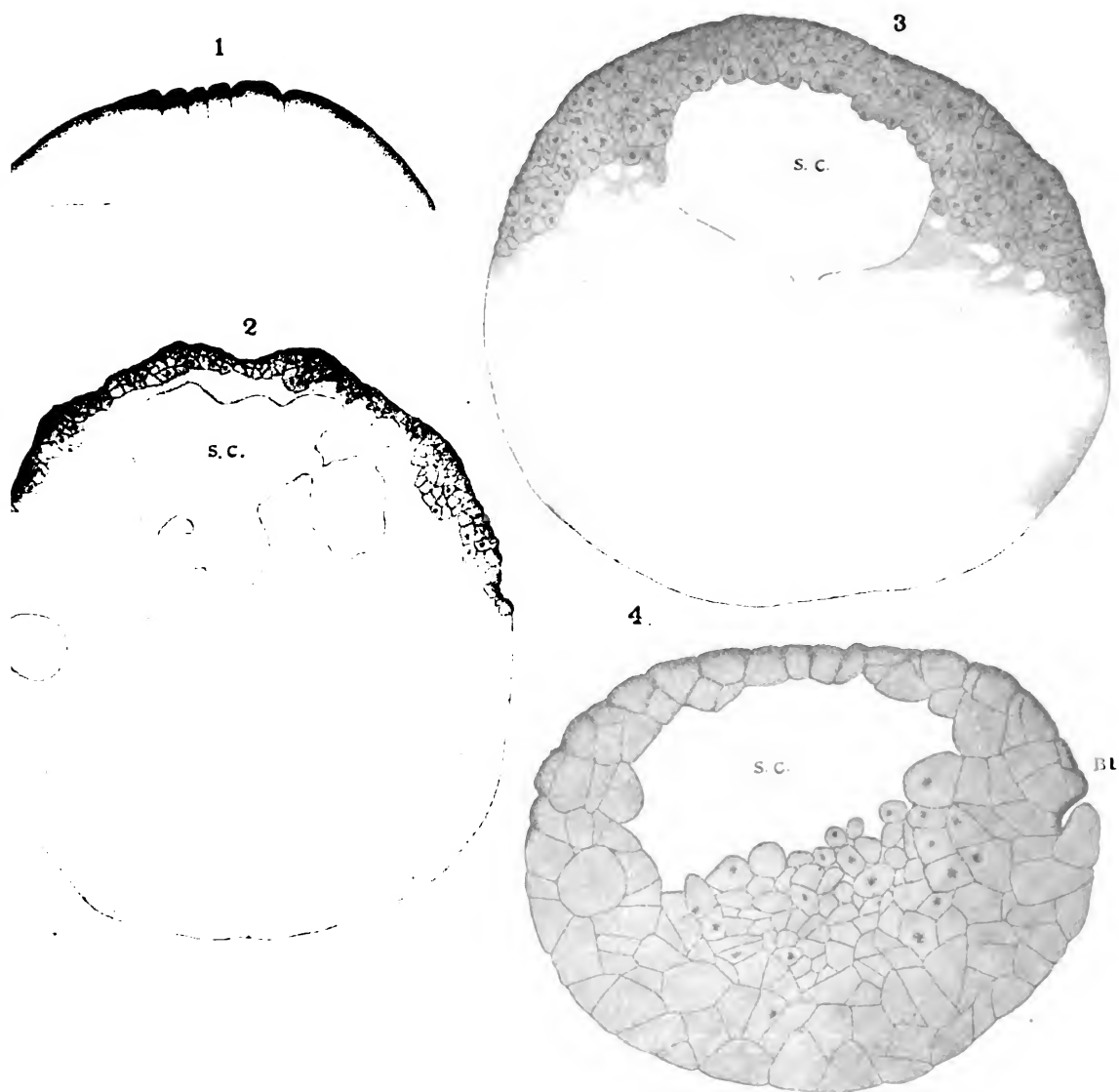
Fig. 1. Part of a section of an egg that was subjected to a temperature of 34–35°C. for one-half of an hour before cleavage began.

Fig. 2. A section of an egg that was exposed to a temperature of 35–36° C. for three quarters of an hour when it was two-cell stage. S.C., segmentation cavity.

Fig. 3. A section of an egg that was subjected to a temperature of 33–35° C. for two hours after the first cleavage plane had appeared.

Fig. 4. A section of an egg that was subjected to a temperature of 31–33° C. for three and one-half hours when it was in the ...-cell stage of development. Bl. blastopore.

however, the eggs of the control sets appeared to be fully as far advanced in development as were the eggs that had been subjected to a higher temperature. Increase in the rate of development is, therefore, but the immediate effect of heat, and after the eggs are brought into a lower temperature they develop at the same, or a lower rate, than the eggs of the control set.

III. Experiments on Eggs in Early Cleavage Stages.

Experiment 6. — On April 17, a lot of about fifty eggs in the 2-cell stage of development was exposed to a temperature of 31–33° C. At the end of one and one-half hours, part of the eggs were removed. They were then in the 8–16-cell stage. The later development of these eggs was perfectly normal in every respect.

The rest of the eggs of this lot remained in the heated chamber for two hours. All of these eggs developed normally during the early cleavage and gastrulation stages; but later a few embryos were found with shortened medullary folds and a large yolk plug at the posterior end of the body. This form of abnormality is very common among embryos that have been injured by exposure to heat.

Experiment 7. — As the first cleavage plane was appearing, a lot of about fifty eggs was subjected to a temperature of 35–36° C. for three quarters of an hour. All of the eggs were segmenting in a very abnormal manner when they were transferred into water at the room temperature, and none of them ever gastrulated. Fig. 2 shows a median section through one of these eggs. With the exception of the layer of small cells bordering the outer surface of the upper hemisphere, the entire substance of the egg is seen to be unsegmented and to have a number of different sized vacuoles scattered through it. A large, irregularly shaped cavity fills the greater part of the upper hemisphere of the egg. This cavity is much larger than the segmentation cavity in a normally segmenting egg, and it appears to be formed of the true segmentation cavity and several large vacuoles which have come to open into it.

Experiment 8. — On April 22, a lot of eggs in the 2-cell stage was exposed to a temperature of 35–36° C. for one hour. When

removed from the chamber the eggs were in the 8-cell stage, but development stopped at this point and all of the eggs were dead inside of twenty-four hours.

Experiment 9. — In this experiment, eggs in the 2- and in the 4-cell stages of development, were subjected to a temperature of 33–35° C. for a period of two hours.   At the end of this time the eggs were segmenting very irregularly in the upper hemisphere and no cleavage planes were visible in the yolk portion of the egg. A section through one of these eggs (Fig. 3) shows the entire upper hemisphere divided into a mass of small cells containing a considerable amount of pigment which is, for the most part, collected in the middle of the cell around the nucleus.   The first cleavage plane has cut only partially through the yolk portion of the egg, as its progress was evidently stopped at the beginning of the experiment.   There are no nuclei in the yolk portion of the egg, and the many vacuoles show the injurious effects of the heat.   The mass of small cells in the upper hemisphere forms a sort of cap on the unsegmented yolk and make it appear as if the segmentation of the egg was meroblastic.   This same sort of abnormal cleavage has also been obtained by Hertwig (1, 2).

According to the experiments in this series, eggs in the early cleavage stages can endure exposure to a temperature of 31–33° C. for a longer period than can the unsegmented egg ; yet they are permanently injured by even a short immersion in water at a temperature of 35°.   The maximum temperature for these eggs, therefore, is not greater than that for the unsegmented egg. Hertwig (4) has found that the maximum temperature for the eggs of *Rana fusca* in the 8-cell stage of development is 26–28°, which is 3–4° higher than that for the unsegmented egg.

IV. Experiments on Eggs in Late Segmentation and Early Gastrula Stages.

Experiment 10. — On April 18, fifty eggs in the 32–64-cell stage of development were kept at a temperature of 31–33° C. for two hours.   Subsequently all of the eggs developed into normal embryos and at about the same rate as did the eggs of the control set.

Experiment 11. — Another set of fifty eggs from the same bunch as the eggs used in experiment 10, was subjected to a temperature of 31–33° C. for three hours. The late segmentation and early gastrulation stages of all of these eggs seemed to be perfectly normal. Two days after the experiment was made, 38 of the eggs were dead, the blastopore not having closed in any case. Of the remaining eggs four only were normal, the rest had a large yolk plug at the posterior end of the body.

Experiment 12. — Twenty-five eggs from the same lot as those used in the two preceding experiments remained in water at a temperature of 31–33° C. for three and one-half hours. Fifteen of the eggs died in the blastula stage. The blastopore appeared in the other ten eggs, but in many cases it was in an unusual position at the equator of the egg. When the dorsal lip of the blastopore was forming in these eggs, the circular blastopore was already beginning to close in the control set of eggs, therefore, in this instance, the heat retarded instead of increased the rate of development of the eggs. In none of the eggs of this set did the blastopore ever become circular, and all of the eggs were dead two days after the experiment was made.

Fig. 4 shows a section of one of these eggs preserved when the blastopore appeared in surface view as a short, straight line at the equatorial zone. The dorsal lip of the blastopore rarely, if ever, comes in as high up as the equator in eggs that are developing normally ; but it sometimes occupies an unusual position in eggs that have been subjected to abnormal conditions. Morgan (5) has found the blastoporic rim above the equatorial zone in eggs of *Rana palustris* that have been subjected to intense cold. In Fig. 4 the archenteron appears as a shallow depression with its dorsal wall formed of heavily pigmented cells as is normally the case. The inner end of the archenteron, instead of turning up towards the black pole as it would do in a normal egg, here projects downward towards the yolk pole. The most interesting fact shown by the section is that the normal position of the large and of the small cells of the egg is completely reversed. In normally gastrulating eggs, the roof of the segmentation cavity is formed of two to three layers of small, pigmented cells, while the ventral wall is composed entirely of large

yolk cells that contain little, if any, pigment. In this egg, however, the upper wall of the segmentation cavity is made up of a single layer of heavily pigmented cells which are fully as large as any other cells in the egg. Below the segmentation cavity, a portion of the yolk is divided into a number of small cells, many of which contain pigment massed around the nucleus. Some of these cells are rounded and seem to lie free in the segmentation cavity, an appearance also noted by Hertwig (4) in eggs of *Rana fusca* that were exposed to a temperature of 29–35° C. after having reached about the 100-cell-stage of development.

Morgan has also noted the relatively large size of the cells in the upper hemisphere of gastrulating eggs of *Rana palustris* that had been subjected to cold. He suggests that this increase in the size of the cells " may be due in part to the absorption of water by the individual cells," and he adds that, " even if this is the case the cells are fewer in number than in a normal egg beginning to gastrulate." In the figure shown by Morgan, the cells of the lower hemisphere are all considerably larger than those of the upper hemisphere; the egg, therefore, must have been much more normal than the one from which Fig. 4 was drawn.

It is evident, in the case of the egg shown in Fig. 4, that the increased temperature did not injure the yolk region or retard its development as is usually the case in these experiments; on the contrary, it is the segmentation of the upper hemisphere that has been delayed, while the segmentation of the lower portion of the egg has continued. No egg in this set of experiments developed much beyond the stage represented by Fig. 4, and each of the ten eggs that were sectioned showed abnormalities of the same general character.

Experiment 13. — On April 26, about seventy-five eggs in the late blastula stage were subjected to a temperature of 33–35° C. A part of the eggs were removed at the end of one and one-half hours and they all developed into normal embryos.

A second portion of the eggs was exposed to this temperature for two and one-half hours. All of these eggs developed into normal embryos, although somewhat more slowly than did those of the control set.

A third part of the eggs remained at the temperature of 33–35° for three and one-half hours. These eggs were all dead when removed from the influence of the heat.

Experiment 14. — A number of eggs in the blastula stage were exposed to a temperature of 36–37° C. on April 26. Some of the eggs were removed from the chamber at the end of one-half of an hour. The eggs did not appear to be injured in any way by the experiment and all developed normally.

A second portion of the eggs from the above lot remained at this temperature of 36–37° C. for three quarters of an hour. All of the eggs gastrulated normally, but about half of them died before the blastopore closed. When sectioned these eggs showed no abnormalities. The rest of the eggs became normal embryos, although developing very slowly. The medullary folds had closed in the eggs of the control set when they were only beginning to unite in the eggs that had been subjected to the increased temperature.

The remaining eggs of this lot were removed to room temperature at the end of one hour. Although the eggs did not appear to be dead ·when they were examined, they did not gastrulate and none of them were alive the day following the experiment.

Experiment 15. — Twenty eggs in late segmentation stages were subjected to a temperature of 40–42° C. for one quarter of an hour. Development was at once stopped by the heat, and all of the eggs were killed.

Experiment 16. — When the dorsal lip of the blastopore was just appearing, a lot of about twenty eggs was put into water at a temperature of 33–35° C. and left there for three hours. All of the eggs continued to develop somewhat more slowly than the eggs of the control set and all became normal embryos.

Experiment 17. — On April 24, a lot of eggs in early gastrulation stages was kept at a temperature of 35–37° C. for one hour. In all of the eggs the lateral and ventral lips of the blastopore formed in the normal manner, but development stopped at this point and the eggs died. No abnormalities were detected when sections were made of several of these eggs.

Experiment 18. — Eggs in early gastrulation stages were exposed to a temperature of 37–38° C. on April 24. A part of the eggs were removed at the end of one quarter of an hour. None of these eggs seemed to be injured in any way by the high degree of heat to which they had been subjected and all developed, somewhat slowly, into normal embryos. The rest of the eggs in this lot remained at the temperature of 37–38° C. for one hour. They were all dead when removed to room temperature.

The results of the experiments in this series show that eggs in the 32–64-cell stage cannot withstand a temperature of 31–33° C. for a much longer period than can eggs that have just begun to segment. The maximum temperature to which eggs can be subjected without injury is practically the same for unsegmented eggs and for those in early cleavage stages, although eggs in the later stages can remain at this temperature for a somewhat longer period and still develop normally.

Eggs in late cleavage stages have a much greater power to withstand high temperature than have eggs in the earlier stages of development, as they will develop normally after exposure to a temperature of 36–37° C. for one-half of an hour. The maximum degree of heat that can be endured without injury is still higher for eggs in the gastrula stages, as they become normal embryos after being subjected to a temperature of 37–38° C. for one quarter of an hour.

The experiments described above are summarized in the following table. The number of the experiment is given in the first column ; the condition of the eggs when the experiment was begun in the second column ; the temperature to which the eggs were subjected in the third column; followed in the next two columns by the duration of the experiment and a brief statement of the results.

The results of these experiments are very similar to those obtained by Hertwig (1–4) in his study of the effects of heat on the development of the eggs of various species of frogs ; and the abnormalities produced resemble, in many respects, those which Hertwig has described and figured. When the unsegmented eggs of *Bufo lentiginosus* are subjected to a temperature that

TABLE I.

No. of Exp.	Condition.	Temperature.	Time.	Result.
1	unsegmented.	28–30° C.	2½ hrs.	Normal development.
2	"	30–32	¾ "	Normal development.
2	"	"	1 "	Four eggs developed normally; the rest died or became abnormal.
2	"	"	2½ "	Most of the eggs died in the blastula stage; a few gastrulated but did not develop further.
3	"	32	2 "	All died in the blastula stage.
4	"	32–33	½ "	Normal development.
5	"	34–35	½ "	Irregular cleavage, no gastrulation.
5	"	"	1 "	Eggs killed.
6	2 cell.	31–33	1½ "	Normal development.
6	"	"	2 "	Most of the eggs developed normally.
7	"	35–36	¾ "	Abnormal cleavage, no gastrulation.
8	"	"	1 "	Development stopped at the eight-cell stage.
9	2–4 cell.	33–35	2 "	Abnormal cleavage, no gastrulation.
10	32–64 cell.	31–33	2 "	Normal development.
11	"	"	3 "	Four normal embryos; the rest of the eggs died or became very abnormal.
12	"	"	3½ "	All of the eggs became abnormal, none of them developed into tadpoles.
13	Late seg.	33–35	1¼ "	Normal development.
13	"	"	2½ "	Normal development.
13	"	"	3½ "	Eggs killed.
14	"	36–37	½ "	Normal development.
14	"	"	¾ "	A few of the eggs developed normally, most of them died in the gastrula stage.
14	"	"	1 "	Eggs killed.
15	"	40–42	¼ "	Eggs killed.
16	Early gastrula.	33–35	3 "	Normal development.
17	"	35–37	1 "	Development stopped when the blastopore was closing in.
18	"	37–38	¼ "	Normal development.
18	"	"	1 "	Eggs killed.

stops their development before gastrulation begins, sections of the eggs show, in many cases, that the greatest injury has been produced in the yolk portion of the egg which is frequently vacuolated and not segmented although the upper part of the egg has divided into a large number of small cells. Hertwig has noticed the same phenomenon in some of his experiments, and in explanation he states as follows: "Dass Froscheier bei erhöhter Temperatur zunächst partiell geschädigt werden und eventuell absterben, ist offenbar auf die verschiedene Organisation der animalen und vegetativen Hälfte der Dotterkugel zurück-

zuführen. Die animale Hälfte der Dotterkugel ist reicher an Protoplasma und steht in höherem Masse unter der Herrschaft des Zellkerns. Unter der normalen Wechselwirkung von Protoplasma und Kern können aber Schäden, welche eine Zelle erlitten hat, wie durch verschiedene Experimente festgestellt worden ist, wieder rückgängig gemacht werden. In dieser Beziehung findet sich die vegetative Hälfte der Eikugel unter ungünstigeren Bedingungen. Denn hier ist das Protoplasma nicht nur spärlicher zwischen den Dotterplättchen vertheilt, sondern ist auch am ungethielten Ei mehr dem Einfluss des Zellkerns, der in der animalen Hälfte liegt, entrückt; später, nach Ablauf der ersten Furchungsstadien sind die Theilstücke vielmals grösser als die aus der animalen Eihälfte entstehenden Zellen."

When the injurious effects of the heat are not manifested until the eggs gastrulate, Hertwig (3) finds, in *Rana fusca*, that the abnormalities produced are of two sorts : First, those with a large yolk plug in the posterior region ; second, those with deformed heads. In all of my experiments on *Bufo*, the abnormal tadpoles, with but very few exceptions, were of the first sort described by Hertwig. In some cases the development of the eggs stopped when the medullary folds were forming and a large yolk plug was found in the mid-dorsal region ; in three cases only was the defect in the anterior part of the embryo. My results are more in accord with Hertwig's experiments on *Rana esculenta* than with those on *Rana fusca*, as in his experiments on the former species he obtained a much smaller number of spina bifida embryos than of those with a large yolk plug at the posterior end of the body.

Hertwig (4) finds that the optimum temperature for the development of *Rana fusca* is 20° C. for the unsegmented egg, and that this optimum rises gradually to 24° C. for eggs in later stages of development. He adds : " Offenbar hängt diese Erscheinung damit zusammen, dass mit der Vermehung der Zellen die Kernsubstanz im Verhältniss zum Protoplasma immer mehr zunimmt und dass so das Protoplasma in höheren Masse ihrem Einfluss unterworfen ist." The optimum temperature for the unsegmented egg of *Bufo lentiginosus* is undoubtedly higher than that for *Rana fusca*, and it is probably somewhere near 28°

C.   This optimum in increased 2–3° for eggs in later stages of development.

In another set of experiments on *Rana fusca*, Hertwig (4) finds that the maximum temperature to which the unsegmented eggs can be subjected without suffering any injury is 23–24° C., while this maximum is increased to 30° C. for eggs in the late segmentation stages.   The maximum temperature for unsegmented eggs of *Rana esculenta* Hertwig finds to be 33° C.   This is also the maximum temperature I have found for unsegmented eggs of the toad, although eggs in the blastula stage can endure a temperature of 38° C. for a very short time.

Morgan has noted that the blastula stages of *Rana palustris* can endure extreme cold much better than can eggs in the 2–4-cell stages, and he also finds that the eggs of *Rana temporaria* which are laid very early in the spring, can survive the tempera- of freezing water for several days.   This temperature would very soon kill eggs of *Rana palustris* which are deposited much later than are the eggs of *Rana temporaria*.

While the eggs of all of these species of *Anura* can withstand a wide range of temperature without injury, there appears to be an adaptation to temperature corresponding to the different periods at which the eggs are deposited.   *Rana fusca* and *Rana temporaria* lay their eggs very early in the spring when the water is often at the freezing point ; and the eggs of these two species can stand cold much better than can the eggs of *Rana palustris* and *Rana esculenta* which are laid considerably later.   Although the eggs of *Bufo lentiginosus* are laid but little later than are those of *Rana palustris*, they are usually deposited in shallow pools of water exposed to the direct rays of the sun.   They must, therefore, often be subjected to a comparatively high degree of heat during the course of their development.

BRYN MAWR COLLEGE,
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THE EFFECTS OF SOME AMIDO–ACIDS ON THE DEVELOPMENT OF THE EGGS OF ARBACIA AND OF CHÆTOPTERUS.

HELEN DEAN KING,

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY.

In 1909, Mathews published a short account of some experiments which he made to ascertain the effects of various amido-acids on the development of the eggs of *Arbacia*. The results of these experiments have considerable theoretical interest, since they seem to show that the course of embryonic development can be determined, to a greater or a less extent, by these products of protein digestion.

While I was working in the Marine Biological Laboratory at Woods Hole, Mass., in the summer of 1909, Dr. Mathews kindly furnished me with a number of amido-acids in order that I might repeat and extend his experiments and make a detailed study of the different types of larvæ that might be obtained. As it seemed worth while to determine whether amido-acids can alter the course of development in various kinds of eggs or whether they have a specific action on the eggs of *Arbacia*, the experiments were carried beyond the limits originally intended and were made with the eggs of an annelid, *Chætopterus pergamentaceus*, as well as with the eggs of the sea-urchin, *Arbacia punctulata*.

In addition to cystin, leucin and tyrosin, the three amido-acids which Mathews used in his experiments, both kinds of eggs were subjected to the action of glutamic acid, aspartic acid, asparagine, glycocoll and alanin. In each series of experiments eggs from two or more females were thoroughly mixed and then artificially fertilized in sea-water. As soon as the polar bodies had been

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extruded, approximately equal portions of the eggs were transferred into finger bowls which contained 100 c.c. of the solution to be tested. As a control by which to judge of the effects of the solutions, one portion of the eggs was allowed to develop in 100 c.c. of normal sea-water. The various experiments were made in a similar manner and the eggs were kept under like conditions of light and of temperature during their development in order that the results of the experiments might not be affected by environmental conditions other than those that were being studied.

A. EXPERIMENTS WITH THE EGGS OF Arbacia punctulata.

As the breeding season of *Arbacia* is near its close the latter part of July, only a small number of eggs suitable for experimental purposes could be obtained. All of the eggs used were presumably in a normal physiological condition, as at least 90 per cent. of those in the control cultures developed in a normal manner and became plutei.

In each series of experiments observations were made at frequent intervals on the living embryos. These observations were later supplemented by a microscopic study of various lots of material that had been fixed in corrosive sublimate and stained with Heidenhain's iron-hæmatoxylin or with Delafield's hæmatoxylin followed by eosin.

Cystin ($C_6H_{12}O_4N_2S_2$).—As this substance is very insoluble in cold sea-water, the solution used in the first experiment that was made was prepared in the following way: A quantity of the pure crystalline salt was placed in a flask of sea-water heated to 40° C. The mixture remained at this temperature for one half hour and was then sealed and set aside. After three days the solution was filtered, to remove the undissolved cystin, and used within a few hours.

A lot of *Arbacia* eggs was fertilized at 11.45 A.M. on the morning of July 14, 1909, and a portion of them was placed in the saturated solution of cystin at 12.15 P.M. These eggs were found to be segmenting in a normal manner when division of the eggs in the control culture took place at 12.50 P.M., and for some hours the eggs of both cultures seemed to be developing at about

the same rate. If the cystin had any effect on the segmentation it was too slight to be detected either in the living eggs or in preserved material.

On the morning of July 15, both cultures contained many living embryos; those of the control were well-developed gastrulæ that were swimming at the surface of the water in a normal manner; those in the cystin solution were decidedly smaller than the control larvæ, and most of them were swimming at the bottom of the dish. Thirty hours after the experiment was started all of the larvæ in the cystin solution were dead, although the larvæ in the control culture were still in good condition. Preserved material showed that the development of the eggs that had been subjected to the action of the cystin solution took place in a perfectly normal manner, although it was somewhat slower than that of the eggs in the control lot.

Mathews found that cystin produced a decided acceleration in the development of the eggs of *Arbacia*, which was apparent from the fourth division on. The solution that he used was made as follows: "One hundred centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish." As a solution made in this way is undoubtedly much weaker than that employed in my first experiment, it seemed probable that the opposing results obtained by Mathews and myself might be due to the difference in the strength of the solutions to which the eggs were subjected. The experiment was therefore repeated with a different lot of eggs, the solution of cystin that was used being prepared in the manner described by Mathews.

In this experiment, also, the development of the eggs appeared to progress at about the same rate in both the cystin culture and in the control. Some of the eggs in the cystin solution seemed to segment much more rapidly than others, and a very few of them developed at a faster rate than the major portion of the eggs in the control culture. A careful comparison between the two cultures, made at intervals of about one half hour during the

entire day, failed to show any marked acceleration in the development of the great majority of the eggs in the cystin solution. Twenty hours after the experiment began swimming larvæ were found at the surface in both cultures, so in this instance the development of the blastulæ was not retarded by the cystin. The solution was ultimately harmful, however, as all of the larvæ in the cystin culture died within thirty-six hours, while those of the control developed into plutei that lived for several days. No unusual types of larvæ were noted among the living forms, and none were found in microscopic preparations of the older embryos.

The *Arbacia* eggs with which Mathews experimented were undoubtedly in a very different physiological condition from those that I used, for Mathews states that in the control lots for his experiments "hardly a pluteus was to be found and these few were generally abnormal." In both of my control cultures the great majority of the eggs formed normal plutei that lived for some days. With such a great difference in the lots of eggs experimented upon it is not surprising that the results do not agree, since the reaction of eggs to any external stimulus depends, to a considerable extent, upon the particular physiological conditions existing in the eggs at the time that the stimulus is applied.

Leucin ($C_6H_{13}NO_2$).—By the use of a weak solution of "impure leucin" Mathews changed the course of development of the eggs of *Arbacia* so that many of the embryos were totally unlike *Arbacia* larvæ. "In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish bipinnaria. . . . Another form was perfectly spherical with a single ciliated band about the middle. It looked in its external form like a small trochophore." Unfortunately, it was not possible to obtain any of the impure leucin with which Mathews produced these remarkable forms of *Arbacia* larvæ, and the leucin with which I experimented was presumably pure.

Solutions of various strengths (2, 1, $\frac{1}{4}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on batches of eggs that were fertilized at 11.30 A.M. on the morning of July 16, 1909. The eggs in all of the cultures began segmenting at the same time as those in the control lot,

but the stronger solutions very soon proved toxic and greatly retarded development. None of the eggs in the 2 per cent. solution of leucin had developed beyond the 2-cell stage at the time that the great majority of the eggs in all of the other solutions, as well as in the control, were in the 8-cell stage. A solution of this strength, however, does not kill the eggs quickly, as twenty hours after the experiment began this culture contained a few ciliated larvæ that were much smaller, and less active, than those of the control lot. Within twenty-four hours all of the larvæ in the 2 per cent. solution of leucin were dead.

A microscopic examination was made of a large number of eggs taken from the 2 per cent. solution of leucin at different stages in their development. Many of the young eggs were abnormal in that there was an irregular distribution of the chromosomes to the poles of the segmentation-spindle or a very unequal division of the blastomeres. Such abnormal eggs evidently died before reaching the blastula stage, as nearly all of the older embryos that were examined were normal although somewhat smaller than those of the control culture. A few abnormal blastulæ were found among the older larvæ, but as these larvæ showed only such irregularities of form as may be found in individuals of almost every control culture of *Arbacia* larvæ developing in a small amount of sea-water under laboratory conditions, they could not be considered as due to the specific action of the leucin in changing the course of development.

The eggs in the 1 per cent. solution of leucin began to show the injurious effects of the solution after the first hour, and from this time on their development, although normal, lagged behind that of the control: the weaker solutions had apparently no effects on the early segmentation. The blastulæ in the control culture began moving about fifteen minutes sooner than the larvæ in the other cultures, so evidently all of the leucin solutions retarded development somewhat after the first two or three hours. Plutei that seemed perfectly normal, and that lived for several days, developed in all of the weaker solutions. An examination of a considerable number of these embryos, preserved at various stages in their development, failed to show any larvæ that were in any way comparable to the unusual types that Mathews obtained with impure leucin.

A second experiment was made with leucin on July 24, 1909. In this instance a solution of the strength of ½ per cent. was employed, since stronger and weaker solutions do not alter the course of development. From the beginning of the experiment the segmentation of these eggs lagged behind that of the eggs in the control lot, and the retardation in development was fully as great as that produced by the 1 per cent. solution of leucin in the former series of experiments. Later the development of these eggs progressed at a more normal rate, and after seven hours the embryos appeared nearly as well developed, and fully as vigorous, as those in the control. The next morning larvæ were swimming at the surface in both cultures, but those in the leucin solution soon dropped to the bottom of the dish and began to disintegrate. Microscopic preparations showed that the very great majority of these larvæ were normal in every respect.

Mathews states that in the summer of 1908, when his experiments were made, the sea-urchin eggs showed in many instances the remarkable peculiarity, recorded by Mathews and Whitcher ('03), that "a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei." The experiments which Mathews made to test the action of amido-acids on the development of the eggs of *Arbacia* were made therefore, wholly or in great part, on eggs that were in a peculiar physiological condition when experimented upon: whether they could be considered as normal is doubtful. The unusual types of larvæ that Mathews obtained by treating eggs with a weak solution of impure leucin were probably due to abnormal or unusual conditions existing in the eggs at the time of their fertilization, and not to the specific actions of leucin in changing the course of development. The effects of leucin on eggs of *Arbacia* that are in a normal physiological condition when fertilized depends chiefly upon the strength of the solution used: a strong solution retards development and causes the early death of the embryos; a weak solution permits of normal development at first and is toxic only after many hours.

Tyrosin ($C_9H_{11}NO_3$).—This substance is not very soluble in cold sea-water, and in order to obtain a solution of sufficient strength one gram of tyrosin crystals was put into 100 c.c. of sea-water and

the mixture brought to the boiling point. The solution was then cooled to laboratory temperature, filtered, and used at once.

The early development of the eggs used in this experiment was normal, although slightly delayed. After twenty hours ciliated larvæ were present in great number in the solution, but they were moving feebly and beginning to show degenerative changes. Prepared material showed that tyrosin had retarded the development of the eggs but produced no abnormalities. These results agree with those obtained by Mathews in a similar experiment.

Glutamic Acid ($C_5H_9NO_4$).—Various solutions of this substance (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on the eggs of *Arbacia*, and all of them proved to be injurious from the beginning of the experiment. The eggs placed in the stronger solutions (1 and $\frac{1}{2}$ per cent.) were killed at once. A few of the eggs subjected to the action of the $\frac{1}{10}$ per cent. solution began to segment in a normal manner, but none of them developed beyond the early stages of segmentation. The eggs in the $\frac{1}{30}$ per cent. solution continued to live for some time, but their development was very greatly retarded and stopped entirely when the gastrula stage was reached. Preparations of these eggs showed that the effects of the glutamic acid was to check development, not to produce unusual types of larvæ.

Aspartic Acid ($C_4H_7NO_4$).—This substance has a more deleterious action on the eggs of arbacia than has glutamic acid. All of the eggs placed in a 1 per cent. solution and in a $\frac{1}{2}$ per cent. solution were killed at once; those subjected to the action of a $\frac{1}{10}$ per cent. solution did not develop beyond the 2-cell stage. A solution of the strength of $\frac{1}{30}$ per cent. allowed a considerable number of the eggs to develop to the blastula stage, but segmentation was very irregular and much slower than that of the eggs in the control culture.

Preparations of various lots of eggs that had been treated with aspartic acid solutions showed abnormal conditions not found in any of the *Arbacia* eggs subjected to the action of other amido-acids. Most of the eggs that had been subjected to the action of a $\frac{1}{10}$ per cent. solution of aspartic acid for four hours before fixation were found to be still unsegmented, and many of them had been entered by several spermatozoa. Only one sperm-

nucleus had fused with the egg-nucleus, however, and the segmentation-spindle that was formed usually appeared normal, although in many cases it occupied a very eccentric position close to the periphery of the egg. All of the accessory spermatozoa at this time were in the form of a small, rounded nuclei that were scattered throughout the cytoplasm.

The $\frac{1}{30}$ per cent. solution of aspartic acid had a different action on different eggs, depending, doubtless, upon the condition of the eggs when they were placed in the solution. Five hours after the experiment was begun about one fourth of the eggs were still unsegmented; some of the eggs were just beginning to segment; while others were in later stages of segmentation, and the cleavage planes were coming in very irregularly in many cases. A very few eggs had reached the blastula stage at this time, but they were not as well developed as the eggs in the control lot. After twenty-two hours the number of eggs that had reached the blastula stage was found to be consideraly increased. Development had been checked by this time, however, and the greater number of larvæ appeared as more or less irregular masses of cells that were beginning to disintegrate.

Preparations of this material showed many cases of polyspermy. Some of the unsegmented eggs contained a large multipolar segmentation-spindle formed, evidently, by the fusion of several sperm-nuclei with the egg-nucleus: other eggs contained a segmentation-spindle of the normal size with the chromosomes very unequally distributed to the spindle poles. The condition of these eggs greatly resembled that which O. and R. Hertwig ('87) found could be induced in fertilized echinoderm eggs by subjecting them to the action of various chemical substances which prevented their normal development.

Asparagine ($C_4H_{10}N_2O_4$).—This amide of aspartic acid proved to be far less injurious to the eggs of *Arbacia* than did the latter substance, when used in solutions of the same strength (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.). The great majority of the eggs in all of the cultures began to segment at the normal time and in a normal manner. After two hours the eggs in the 1 per cent. solution showed evidence of retarded development, but the eggs in all of the other solutions developed at a normal rate for some hours.

Twenty-four hours after the experiment began, ciliated larvæ were present in great numbers in all of the solutions, but they all died many hours before the death of the larvæ in the control culture.

Glycocoll ($C_2H_5NO_2$).—This substance, which is the simplest of the amido-acids, was much less harmful to the eggs of *Arbacia* than were any of the other amido-acids used in these experiments. During the first twenty-four hours the development of the eggs did not appear to be affected in any way by the solutions used ($1, \frac{1}{2}, \frac{1}{10}$ and $\frac{1}{30}$ per cent.), but during the second day the embryos began to show degenerative changes, and all of them died about fifty hours after the experiment began. Sections of these eggs fixed at various stages of development merely confirmed the observations on the living forms, as no unusual types of larvæ were found.

Alanin ($C_3H_7NO_4$).—This amido-acid dissolves readily in cold sea-water, and it was used in solutions of the following strengths: $2, 1, \frac{1}{2}, \frac{1}{10}$ and $\frac{1}{30}$ per cent. The stronger solutions ($2, 1$ and $\frac{1}{2}$ per cent.) retarded development from the beginning: the weaker solutions had no apparent effects on the segmentation of the eggs. After twenty-four hours each of the solutions contained a large number of swimming larvæ, and only those in the 2 per cent. solution showed any evidence of retarded development. The embryos in all of the cultures died some hours before the death of the control larvæ, so weak solutions of alanin cannot be considered as favorable media in which to rear the eggs of *Arbacia*. Preserved material showed no abnormalities worthy of note at any stage of development.

All of the amido-acids used in this series of experiments with the eggs of *Arbacia* proved to be toxic, the injurious effects of any substance depending very largely upon the strength of the solution used. In no case was the course of development altered in a definite direction, except in the very young eggs and in these the abnormalities produced were of the types commonly found when fertilized eggs of the sea-urchin are treated with various chemical solutions.

B. EXPERIMENTS WITH THE EGGS OF *Chætopterus pergamentaceus.*

As the eggs of *Chætopterus* could be obtained in considerable numbers at Woods Hole in the summer of 1909, experiments were made to study the influence of amido-acids on the early development of this annelid, in the hope that some definite alterations in development might be produced comparable to those obtained by Loeb ('01) and by Lillie ('02) when eggs of *Chætopterus* were treated with potassium salts. Material intended for microscopic study was preserved in Boveri's picric-acetic solution and stained with hæmatoxylin.

Cystin.—On the morning of August 6, 1909, a lot of *Chætopterus* eggs was placed in 100 c.c. of a saturated solution of cystin as soon as the polar bodies had been extruded. The early development of these eggs was slightly accelerated, and swimming larvæ were found in this culture nearly one half hour before any movement could be detected in the control larvæ. The next day the cystin solution was swarming with well-developed trochophores, but they all died about fifty hours after the experiment began. No abnormal embryos were noted at any stages of development and none were found in preserved material.

The experiment was repeated several days later with eggs from another female. The results obtained were practically the same as in the first experiment, since there was more rapid development during the segmentation period. The solution proved to be toxic after thirty hours, however, killing the embryos without producing any alterations in structure.

Leucin.—In one series of experiments this substance was used on the eggs of *Chætopterus* in solutions of the following strengths: $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{80}$ per cent. None of these solutions had any marked effects on the early segmentation of the eggs, but they evidently caused a slight acceleration in development during a later period as the larvæ in all of the solutions began moving some thirty minutes before there was any movement of the control larvæ. Twenty hours after the experiments were started all of the cultures were carefully examined. The majority of the eggs that had been treated with the $\frac{1}{2}$ per cent. solution had stopped their development in the blastula stage, and were lying at the bottom of the dish apparently dead; a very few larvæ were swimming

at the surface of the solution, but they had evidently reached their maximum development and would soon die. The $\frac{1}{10}$ per cent. solution contained a considerable number of swimming larvæ, but these larvæ were not in good condition and plainly showed the injurious effects of the leucin. A large number of ciliated embryos were found in the $\frac{1}{30}$ per cent. solution, and they appeared somewhat further advanced in development than those in the control culture. Degenerative changes appeared in these larvæ in about twenty-four hours, however, and all of them were dead within thirty hours. No unusual types of larvæ were found in preparations of these eggs fixed at various stages in their development.

As it seemed possible that the solutions of leucin employed in the experiments described above might have been too weak to produce any alteration in the development of the eggs, a second experiment was made in which a batch of eggs was subjected to the action of a 1 per cent. solution of leucin. These eggs segmented at the normal time, but two hours later their development was found to be lagging behind that of the eggs in the control culture. After four hours the retardation in development was very marked, and in some instances two or more eggs had fused together. Loeb and Lillie have noted that the fusion of several embryos into giant forms is a phenomenon of frequent occurrence when eggs of *Chætopterus* are treated with potassium salts. In twenty hours all of the larvæ were dead, and so disintegrated that it was impossible to preserve any material fit for study. Sections of eggs fixed in earlier stages of development failed to show any abnormalities except the occasional fusion of two or more embryos.

Tyrosin.—This substance was used on the eggs of *Chætopterus* in a saturated solution which is less than $\frac{1}{10}$ per cent. Only a very few of the eggs had segmented when the first division occurred in the control eggs. After four hours the tyrosin culture showed all stages in development from the unsegmented egg through to late segmentation, the most advanced eggs being apparently at the same stage of development as the eggs of the control. All of the embryos in the tyrosin solution died within twenty-four hours after the experiment was started. Preserved

material showed that tyrosin acts on the eggs of *Chœtopterus* as it does on the eggs of *Arbacia*, causing a marked retardation in development but producing no specific abnormalities.

Glutamic Acid.—Solutions of various strengths (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.) were used, the eggs being placed in the solutions about three quarters of an hour after their fertilization. All of the eggs in the two stronger solutions were evidently killed at once as none of them made any attempts to divide. Some of the eggs in the $\frac{1}{10}$ per cent. solution began to elongate after the solution had acted upon them for one hour, and later many of these eggs took on an irregular shape as if attempting to divide into several cells at the same time. None of these eggs had segmented after five hours, however, so they were all returned to normal sea-water in the hope that they would then be able to continue their development. There was no segmentation of any of the eggs, although they appeared to live for some hours.

Sections of preserved material showed that the segmentation-spindle had formed in many eggs in an apparently normal manner, but that development had been stopped at this point.

Aspartic Acid.—Eggs of *Chœtopterus* fertilized at 10.55 A.M. on August 8, 1909, were placed in solutions of aspartic acid (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{80}$ per cent.) at 11.25 A.M. The eggs in the control culture were segmenting at 11.55 A.M., but no evidence of cleavage could be detected in any of the eggs in the aspartic acid solutions until 1.30 P.M., when a few of the eggs in the $\frac{1}{80}$ per cent. solution began to elongate as if about to divide. A number of these elongated eggs were isolated and carefully watched for some time, but in no case did any division occur. Sections of preserved material showed that some eggs contained a normal segmentation-spindle, while others had a multipolar spindle that occupied an eccentric position close to the periphery. The stronger solutions of aspartic acid killed the eggs before the formation of the segmentation-spindle.

Asparagine.—Solutions of this substance of the same strengths as those used in the experiments with aspartic acid were tested. Normal cleavage began in the eggs of all of the cultures at the same time as in those of the control lot. Observations made at frequent intervals during the next four hours showed that seg-

mentation was progressing in a normal manner and at about the same rate in all of the solutions.

Five hours after the eggs had been fertilized a few larvæ in the $\frac{1}{10}$ per cent. solution were moving slowly: at this time there was no movement of any of the embryos in the other cultures or in the control lot. A weak solution of asparagine, therefore, slightly accelerates the development of the eggs of *Chætopterus*, if it be that an earlier movement of the embryos is indicative of a more advanced stage of development. At the end of the sixth hour the effects of the various solutions were very marked: the embryos in the $\frac{1}{10}$ per cent. solution were moving more actively than those in the control, and they seemed slightly better developed; the larvæ in the other solutions were moving slowly and their development lagged considerably behind that of the control larvæ. After eight hours the larvæ in the 1 per cent. solution were all at the bottom of the dish and evidently dying; no abnormal types of larvæ could be detected among the living forms, and none were found in preserved material that was examined later. The embryos in the other solutions were swimming at the surface after ten hours, but none of them lived more than twenty-four hours.

Glycocoll.—In the strengths of solutions used (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.), this substance did not appear to have any effects whatever on the eggs during the first twelve hours. On the second day the larvæ began dying, and all of them had been killed by the end of the third day.

Alanin.—Batches of *Chætopterus* eggs that had been artificially fertilized at 10.30 A.M. on the morning of August 8, 1909, were put into various solutions of alanin (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11 o'clock. The eggs in all of the cultures, including the control, began segmenting at the same time, and all of them developed at about the same rate during the next two hours. At 3.30 P.M. a number of swimming larvæ were found in the $\frac{1}{10}$ and in the $\frac{1}{30}$ per cent. solutions, but at this time there was no movement of the larvæ in any of the other cultures. At 4.30 P.M. ciliated larvæ were present in great numbers in all of the solutions; but the larvæ in the 1 per cent. solution could move but slowly, and soon all of them sank to the bottom of the dish and disintegrated.

At 9 A.M. on the morning of August 9, the larvæ in the $\frac{1}{2}$ per cent. solution were dying, and a number of giant embryos had been formed by the fusion of two or more of the larvæ: the embryos in the $\frac{1}{10}$ per cent. and in the $\frac{1}{30}$ per cent. solutions were apparently normal and were moving vigorously. All of the larvæ were dead on the morning of August 10, although the trochophores in the control culture were still very active at this time. Preserved material showed no abnormalities worthy of note.

As weak solutions of alanin did not seem to affect the early development of the eggs adversely a second series of experiments was made in which batches of *Chætopterus* eggs were treated with 4 per cent. and with 2 per cent. solutions of alanin as soon a they had extruded their polar bodies.

None of the eggs in the 4 per cent. solution segmented, and sections of preserved material showed that the eggs had been killed before the formation of the segmentation-spindle. When cleavage began in the eggs of the control lot at 11 A.M. a very few of the eggs in the 2 per cent. solution were dividing in an apparently normal manner; in the great majority of the eggs segmentation was very greatly delayed. After four hours only a few eggs had reached the 4-cell stage, and in these eggs the cleavage planes had come in very irregularly. An hour later development had stopped entirely and the eggs were fusing into large, irregularly shaped masses. At this time the eggs were transferred into normal sea-water in the hope that segmentation might be resumed, but although the eggs seemed to live for some hours, none of them developed beyond the 4-cell stage.

In microscopic preparations of eggs that had been in the 2 per cent. solution of alanin for two hours before fixation only a very few normal 2-cell stages were found, and the great majority of the eggs contained a multipolar spindle with the chromosomes very irregularly distributed along the spindle fibres. Material fixed after the solution had acted for five hours showed that only the first cleavage in any of the eggs was normal and that in most eggs development had stopped at this point. Where further division had occurred the blastomeres were very irregular in size and shape, and although hundreds of eggs were examined no stage later than an 8-cell stage could be found.

When multipolar spindles formed in the eggs as a result of their treatment with a 2 per cent. solution of alanin the eggs, apparently, were never able to divide, although there seemed to be a long period during which active and resting stages alternated with each other. In the resting stages the eggs contained either one large, oblong nucleus, or several smaller ones that were more or less irregular in outline. In the active periods one large, multipolar spindle with hundreds of chromosomes scattered about it would be formed, or several small spindles, all more or less irregular, would be scattered throughout the cell. In some of these eggs a number of accessory asters were formed, similar to those that Morgan ('96, '99) found could be produced in the eggs of *Arbacia* and of various other forms by means of salt solutions.

A 2 per cent. solution of alanin produced greater abnormalities in the eggs of *Chætopterus* than did any of the other solutions of amido-acids that were used, but as these abnormalities were of the types that can be produced in different kinds of eggs by treatment with various salts they cannot be considered as the result of any specific action on the part of the alanin.

SUMMARY AND CONCLUSIONS.

With the exception of cystin, which is a sulphur-containing compound, all of the amido-acids used in these experiments are composed of the same chemical elements, yet they differ to a marked extent in their toxic action on developing eggs. Glutamic acid and aspartic acid are by far the most injurious, even a $\frac{1}{30}$ per cent. solution of these substances killing the eggs of both *Arbacia* and of *Chætopterus* at a very early period. Glycocoll, on the other hand, permits of the development of normal plutei and trochophores, and only injures the embryos after twenty-four hours. The other amido-acids used retard development, to a greater or less extent, depending chiefly upon the strength of the solution employed.

A brief summary of the effects of the various solutions of amido-acids on the development of the eggs of *Arbacia* and of *Chætopterus* during the first twelve hours is given in the following table. Ultimately all of the solutions are toxic, even though they appear to favor development during an early period.

TABLE I.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs
Cystin	Saturated	No effects on segmentation; later development retarded.	Development accelerated.
Leucin.	$\frac{1}{30}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{4}$ per cent.	Development very slightly retarded.	. . . .
	$\frac{1}{2}$ per cent.	Development slightly retarded.	Development accelerated at first, but stopped in blastula stage.
	1 per cent.	Development retarded after 1 hour.	Development retarded after 2 hours; embryos fused.
	2 per cent.	Development greatly retarded; a few eggs abnormal.	. . . .
Tyrosin.	Saturated.	Development retarded.	Development retarded.
Glutamic acid.	$\frac{1}{30}$ per cent.	Development stopped in the gastrula stage.	. . . .
	$\frac{1}{10}$ per cent.	Eggs killed in early segmentation.	Eggs lived for some time, but no segmentation.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
Aspartic acid.	$\frac{1}{30}$ per cent.	Development stopped in blastula stage; many eggs abnormal.	Eggs lived for some time, but no segmentation.
	$\frac{1}{10}$ per cent.	Development stopped at 2-cell stage; many eggs abnormal.	Eggs killed at once.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
Aspara-gine.	$\frac{1}{30}$ per cent.	No effects noted.	. . . .
	$\frac{1}{10}$ per cent.	No effects notes.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	No effects noted.	Segmentation not affected; later development retarded.
	1 per cent.	Development retarded after 2 hours.	Segmentation not affected; later development retarded.
Glycocoll.	$\frac{1}{30}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{10}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{2}$ per cent.	No effects noted.	No effects noted.
	1 per cent.	No effects noted.	No effects noted.
Alanin.	$\frac{1}{30}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	Development somewhat retarded.	Segmentation not affected; older embryos fused.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs.
	1 per cent.	Development greatly retarded.	Development retarded after 2 hours.
Alanin.	2 per cent.	Development greatly retarded.	Development retarded; many eggs abnormal.
	4 per cent.	....	Eggs killed at once.

As shown in the above table, all of the stronger solutions of amido-acids that were used had much the same effect on both kinds of eggs experimented upon, but several of the weaker solutions had a much more pronounced action on the eggs of *Chætopterus* than on those of *Arbacia*. Weak solutions of cystin, of leucin, of asparagine and of alanin accelerate the development of the eggs of *Chætopterus* to a noticeable extent, yet none of these solutions have apparently any effect on the early development of the eggs of *Arbacia*. The eggs of *Chætopterus* cannot segment at all when placed in a $\frac{1}{30}$ per cent. solution of aspartic acid, although this solution permits the eggs of *Arbacia* to develop to the blastula stage.

The abnormalities produced in the eggs of *Arbacia* and of *Chætopterus* by various solutions of amido-acids consist chiefly of polyspermy, irregularities in the mitotic figures, variable cleavage, and a fusion of several embryos into giant forms. No embryos were found that showed either the larval characteristics of other forms or marked peculiarities of structure that might be attributed to the specific action of the solution in which they were reared.

The results obtained in these experiments indicate that solutions of amido-acids can alter the rate at which the eggs of *Arbacia* and of *Chætopterus* develop, but that they have no influence whatever in determining the character of the development, when the eggs experimented upon are in a normal physiological condition.

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7. Food as a factor in the determination of sex in Amphibians. 7

From the Writer

FOOD AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

HELEN DEAN KING.

Of the many theories that have been advanced regarding the causes that determine whether an animal shall become male or female, the one that nutrition is a dominant factor in sex determination has received much credence. This theory has been supported by the results of numerous feeding experiments made by different investigators on various classes of animals, and also by statistics compiled by Düsing (5) and others with reference to the proportion of males and females in the human race among the offspring of the rich and of the poor.

Three investigators, Born, Yung and Cuénot, have sought by experimental means to ascertain the relation of nutrition to sex determination in amphibians. Born (1), who was the first investigator in this field, found that in a total of 1,272 young *Rana fusca* that had been well nourished during the larval period, 1209 or about 95 per cent. were females, while in 160 young frogs taken from their natural environment only 52 per cent. were females. From the results of these experiments Born concluded that an abundance of food leads to the development of a greater proportion of females. As several investigators have pointed out, Born's results cannot be considered as furnishing conclusive evidence regarding the influence of nutrition on sex determination, for the methods employed in the experiments did not exclude the possibility that other factors than nutrition influenced the results. No account whatever was taken of the many hundreds of tadpoles that died during the course of the investigations and, as Born himself suggests, there is the possibility that the mortality was greater among the males than among the females. In ascertaining the sex of the young frogs, Born examined the gonads *in toto* and did not make use of sections in any case : if the genital organs were large, the individual was classed as a female ; if the organs were small, the individual

was considered to be a male. Such a method of distinguishing the sexes in young frogs has been found to be unreliable, as at the time of metamorphosis the genital organs are not very well developed and it is often impossible to determine the sex of an individual with any degree of certainty without making a histological examination of the gonads.

The experiments of Yung (13) on *Rana esculenta* were made, primarily, to study the influence of various kinds of food on the development of the tadpoles, but the results seem to furnish positive evidence that the sex of *Rana* is influenced by nutrition. Yung's experiments were carried out with great care, the different lots of eggs being kept under similar external conditions and the food alone differing in the various cases. In considering his results, Yung also failed to take into account the tadpoles that died during the course of the experiments, and he ascertained the sex of only those individuals that underwent metamorphosis. In these experiments the number of females that developed varied from 70 per cent. to 75 per cent. in different cases, the greatest number being found among the lot of frogs that had received only animal food. In a later series of experiments Yung (14) found that in a lot of 100 young frogs that had been fed exclusively on beef, 78 per cent. were females; the number of females was found to be increased to 81 per cent. in a second lot of 100 tadpoles that had been fed on fish; while in a third lot of 100 tadpoles that had received the flesh of frogs as food the number of females was 92 per cent. From an investigation of the sex of 300 young *Rana esculenta* that had developed under natural conditions, Yung concluded that normally the number of females in this species of *Rana* is about 53 per cent. The results of Yung's experiments, therefore, support Born's conclusion that nutrition is a decisive factor in sex determination, an abundance of food leading to the development of a large proportion of females. ·

In a recent paper, Cuénot (3) gives the results of a series of feeding experiments which he made on the larvæ of *Rana temporaria* in order to test the conclusion reached by Born and Yung. Cuénot's results do not agree with those obtained by the earlier investigators, as in two lots of frogs that had been well nourished on animal food he found an excess of males; while in another

. lot of frogs that had been poorly nourished, there was a greater proportion of females.   Cuénot states that, as the results of all of the feeding experiments that have been made on *Rana* are contradictory, it is evident that nutrition is not an absolutely dominating factor in sex determination.   He believes that there is a strong probability that sex is already determined in the egg at the time of deposition.

As Cuénot used comparatively few individuals in his experiments and as his results do not accord with those obtained by Born and Yung, it is obvious that the question of the influence of nutrition in determining the sex of amphibians is still an open one.   It is necessary, therefore, that many more experiments should be carried out along the lines suggested by the work of these investigators.

At the anterior end of the genital organs in the tadpoles of the common American toad, *Bufo lentiginosus*, there is found a small rounded structure, the so-called " Bidder's organ " (Fig. 1, *B*), which is composed apparently of undeveloped ova.   The function of this organ is unknown, and whether it is a rudimentary ovary, as many investigators have maintained, has not as yet been satisfactorily determined.   This body is found in young tadpoles some time before it is possible to distinguish sex ; and it is a permanent organ in the male, disappearing in the female near the end of the second year.   If Bidder's organ proves to be a rudimentary ovary, then the adult male toads are in a sense hermaphrodites, although the same cannot be said of the adult female unless the male elements are present in some form that as yet has not been discovered.   Because, therefore, of a possible condition of hermaphroditism in the young tadpoles, which might seem to indicate that sex is not already determined at this stage of development, *Bufo lentiginosus* was chosen as more favorable material than any common species of *Rana* for an investigation of the influence of external factors on sex determination.   As the tadpoles of *Bufo* are somewhat smaller than those of *Rana* and are easily reared under artificial conditions, they are well adapted for experiments that must, of necessity, extend over a considerable period of time.

The present paper records the results of the first of a series of

experiments which have been planned in the hope that it may be
possible to show whether external factors such as temperature,
nutrition, time of fertilization, etc., have any influence in determin-
ing sex in *Bufo*.   Recent investigations on other forms have
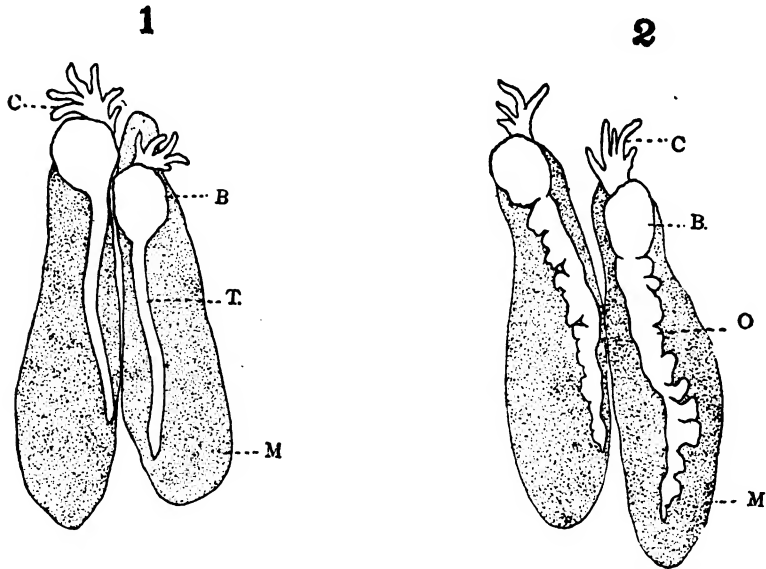
FIG. 1.   Camera drawing of the genital organs of a male toad killed soon after
matamorphosis.   *T*, testis; *B*, Bidder's organ; *C*, corpus adiposum; *M*, kidneys.
FIG. 2.   Camera drawing of the genital organs of a female toad killed soon after
metamorphosis.   *O*, ovary.   Other lettering as in Fig. 1.

seemed to indicate that sex is not influenced by external condi-
tions, but that it is determined either before or during the fertiliza-
tion of the egg.   If this is indeed the case, experiments such as
those that I have in mind will yield only negative results.   But if it
can be shown for even one form that external factors may be
disregarded in considering the question of sex determination,
something will have been gained towards an ultimate solution of
the problem.

METHOD.

In endeavoring to ascertain the part played by any one factor
in sex determination, it is, of course, absolutely necessary that
the influence of all other factors shall be eliminated as far as pos-
sible.   In making the experiments recorded in the present paper,

very great care was taken that all of the individuals being ex-
perimented upon were kept under similar external conditions, the
only factor that was intentionally varied being that of nutrition
whose action it was proposed to study.

Two separate series of experiments were made which, for con-
venience, will be called Series I., and Series II.   The eggs used
in Series I., were laid in the laboratory and normally fertilized cn
the morning of April 13, 1906 ; while those used in Series II.
were laid under similar conditions on April 16, 1906.   Both lots
of eggs were kept in large aquaria until the tadpoles hatched, and
the experiments began in each case five days after the eggs were
laid.

Series I. was started with a total of 1,500 individuals ; but, as
will be explained later, only 1,100 of these can be taken into
account in considering the results.   Eight hundred individuals were
used in the second series of experiments, making a total of 1,900
individuals upon which to base conclusions from the results ob-
tained.   Glass dishes of uniform size were used throughout the ex-
periments, each dish containing, in the beginning, 100 tadpoles.
The dishes were kept together so that the tadpoles were all under
the same conditions of temperature.   The water used was " tap "
water obtained from an artesian well and used for drinking pur-
poses, so presumably it was free from unicellular organisms.
Approximately the same quantity of water was kept in each dish.

Out of the total of 1,900 individuals, only 364, or 19.15 per
cent. died before it was possible to ascertain the sex.   This com-
paratively low rate of mortality during the early stages of develop-
ment I attribute in great part to the fact that the water in the
dishes was never allowed to become foul.   When the tadpoles
were very small the water was changed on alternate days and the
dishes carefully cleaned.   Later, as the tadpoles became larger,
it was necessary to change the water every day.   During some
very warm weather in June when the tadpoles were beginning to
undergo metamorphosis, the water was renewed as often as four
or five times daily.

In *Bufo* the genital organs are apparently much better devel-
oped at the time of metamorphosis than they are in *Rana*, as in
the majority of individuals it is possible to distinguish the males

from the females with absolute certainty without making a his-
tological examination of the gonads. The method used to dis-
tinguish the sexes in very young toads was as follows: the toad
was placed in a flat, shallow dish containing a layer of paraffine
which makes an excellent surface for cutting, and the body cavity
was then opened under a dissecting lens; the kidneys and the
genital organs attached to them were removed by means of small,
sharp knives, and subsequently, under a much stronger lens, the
gonads were examined *in toto*. Figs. 1 and 2 show the differ-
ences between the gonads of the two sexes in young toads that
have recently completed metamorphosis. The testes (Fig. 1) are
at this time about 2 mm. in length, they are relatively narrow,
cylindrical bodies with a smooth outline; the ovaries (Fig. 2),
on the contrary, are usually broader than the testes and they
have an irregular, jagged outline. Bidder's organ (Figs. 1 and
2, *B*) is very prominent in all individuals at this time; but as it
is practically the same size in both sexes, it is of no aid, in dis-
tinguishing males from females.

In order to ascertain whether the external appearance of the
genital organs (as shown in Figs. 1 and 2) is a positive indication
of the sex of the individual, 50 young toads were selected of which
25 had gonads approximately like those shown in Fig. 1, and 25
had gonads similar to those in Fig. 2. The gonads were stained *in
toto* with hæmatoxylin and sectioned. The histological examina-
tion proved conclusively that the external appearance of the
gonads can be relied on to indicate the difference in sex, as
the sections showed unquestionably that there were 25 males in
the one lot and 25 females in the other. At the time of meta-
morphosis the genital organs are not equally well developed in
all individual, however, and occasionally it is impossible to dis-
tinguish the sex of a toad without making use of sections.

All of the tadpoles that died during the course of the experi-
ments were fixed in corrosive-acetic (5 per cent. acetic acid) if
the hind legs were well developed and the sex ascertained, when
possible, by means of sections. A histological examination of the
gonads enables one to ascertain the sex of a tadpole some time
before the front legs have appeared; for, although the germ-cells
may appear similar at this time, the ovary has a central cavity

which is not present in the testis.   Altogether the gonads of about 600 individuals were examined histologically and in only about 50 cases was it impossible to distinguish one sex from the other.

The methods used in carrying out the experiments and in ascertaining the sex of the individuals have been given in considerable detail in order to indicate the precautions that were taken to avoid the most probable sources of error that might have had an influence on the results.

The Normal Proportion of the Sexes in Bufo lentiginosus.

Cuénot has collected the statistics that have been published regarding the normal proportion of the sexes in various species of *Rana*, and his table shows that the number of females varies from 49 per cent. to 86.8 per cent. in different cases.   Pflüger (9) and von Griesheim (7), who have most carefully investigated this subject, find that not only does the proportion of females vary somewhat in lots of frogs taken from different localities, but that there is also a marked difference in the proportion of females in lots of frogs taken from the same locality in different years. The normal proportion of the sexes in *Rana* seems, therefore, to be a variable one depending on the locality and on the year. In the great majority of cases there seems to be a greater number of females than of males, not only among adult frogs but also among the young just after metamorphosis : the excess varies from 1.05 per cent. to 73 per cent. in different cases.

I have not been able to find any statistics regarding the normal proportion of the sexes in other amphibians.   Fischer-Sigwart (6) has noticed an excess of males among *Hyla aborea* during the breeding season, and Boulenger (2) has stated that there is an excess of males among the common European toads, *Bufo vulgaris* and *Bufo clamata :* neither investigator gives any statistics in support of his statement.   For some years past I have been collecting adult toads during the breeding season and also during the summer months, and I have always found an excess of males in this species.   Unfortunately I have kept no records regarding the proportion of the sexes among adults.

In order to determine the relative proportion of the sexes in young toads that have recently completed their metamorphosis

500 individuals were collected one morning from the bank of the
Susquehanna River at Owego, N. Y., during the latter part of
June, 1904. The sex of each individual was ascertained by the
method described above, it being necessary to make a histological
examination of the gonads in only about twenty cases. The
result of the investigation is summarized by hundreds in the fol-
lowing table.

TABLE I.

Number of Individuals.	Males.	Females.
100	51	49
100	44	56
100	46	54
100	48	52
100	52	48
500	241	259

Of the total of 500 individuals, 259 or 51.8 per cent. were
females, and 241 or 48.2 per cent. were males. In *Bufo* the
excess of females among the young seems to be somewhat less
than that among young frogs, as according to an investigation
made by von Griesheim of the sex of 440 young *Rana fusca*, 280
or 63.7 per cent. were females.

Although in the adult state the female toad is noticeably larger
than the male, it is not possible to distinguish the sex of very
young toads by their size alone. Two hundred individuals in this
group were sorted according to size and it was found that, in many
cases, the larger individuals were males. Any variation that may
exist in the size of the individuals at the time of metamorphosis
can probably be attributed to the difference in the amount of food
that the tadpoles were able to obtain.

EXPERIMENTS.

If food is a decisive factor in sex determination, it may be
considered to act in one of two ways : either through the quantity
of nourishment that it affords the organism ; or through its par-
ticular chemical nature as a proteid, a hydrocarbon, etc. An
abundant nutrition is held by many investigators to lead to the
development of an excess of females ; while, on the other hand,
scarcity of food, according to Schenk (10) and others, tends to

produce relatively more males.   Yung maintains that nitroge-
nous food is highly favorable to the development of females ; while
Schultze (11) states that food of this character has no influence
whatever in determining sex.

It was intended, when the experiments began, to test both of
the possibilities mentioned above.   Among the 300 individuals of
Series I., that were poorly nourished the mortality was so great
during the first month of the experiment that it was necessary to
abandon, for the time, the study of the possible influence of mal-
nutrition on sex determination.   The investigations were there-
fore confined to an attempt to ascertain whether an abundant
nutrition or the character of the food received by the larvæ has
any influence in determinating sex.   The lot of 300 tadpoles
which had received little food was therefore discarded, and all of
the remaining individuals received an abundance of the particular
kind of food whose influence was being investigated.

In Series I., 300 tadpoles (Lot A) were fed exclusively on a
meat diet consisting of small pieces of cooked lamb or beef ; 300
tadpoles (Lot B) were nourished on a purely vegetable food con-
sisting of a cooked wheat cereal ; a third set of 300 individuals
(Lot C) received a mixed diet composed of water plants (*Nitella*
and *Spirogyra*) and minute organisms on decayed leaves and bits
of wood taken from a pond in which toads breed each spring.
Lot C presumably received food similar in character to that
normally obtained by amphibian larvæ.

According to experiments made by Danilewsky (4), lecithin has
a marked influence on the development of frog embryos : tad-
poles fed on it show a great increase in size and in weight over
control tadpoles that have not received lecithin as food.   Dani-
lewsky's experiments were not continued until the tadpoles
underwent metamorphosis, and therefore his results do not indi-
cate whether the increase in the size of the tadpoles was due to a
more rapid development or whether it was the direct effect of the
lecithin in producing abnormally large individuals.   As it is con-
ceivable that a more rapid development or an abnormal increase
in size might possibly be factors that would influence the sex of
an individual, a fourth set of 300 tadpoles (Lot D) in Series I.
were fed exclusively on the yolk of hen's egg which, according

to Gautier, contains from 8.43 per cent. to 10.72 per cent. of lecithin. No attempt was made to feed tadpoles on lecithin alone, because in experiments which I made several years ago the mortality among tadpoles that were given lecithin as food was exceedingly great; the individuals dying evidently of starvation, as they were never seen to eat any of the lecithin. Owing to an accident, 100 tadpoles fed on the yolk of egg were killed the second week of the experiment. Lot D, therefore, consisted of only 200 individuals, making a total of 1,100 individuals that are to be taken into account in considering the results of the experiments in Series I.

In order to make possible a comparison between the results obtained in Series I. and those from similar experiments on the eggs of a different female, a second series of experiments were made beginning three days later than those in Series I. These experiments were similar in all respects to those in the first series, except that no attempt was made to investigate the possible influence of a scarcity of food in determing sex, and only 200 tadpoles were used in each lot. Series II. therefore consisted of 800 individuals.

Although detailed observations were made on each lot of tadpoles at intervals of about one week, only the record of Series I. for June 7, will be given. This record will serve to show the differences between the individuals of the various lots that can probably be ascribed to the varied character of the food that the tadpoles received.

Lot A. — The tadpoles fed exclusively on meat were noticeably larger than those fed on any other kind of food. The largest individuals measured 27 mm. in length, thus exceeding, by 3–4 mm., the length of a number of tadpoles of about the same age that had been reared under natural conditions; the smallest individuals in this lot were 19 mm. long and were much larger than many of the tadpoles in the other lots. Many of the meat fed tadpoles had very well developed hind legs at this time, but the front legs had not appeared in any individual as yet. It was noticed that these tadpoles were very much blacker than any of the other tadpoles being experimented upon. A meat diet is evidently as favorable to the development of pigment

in the toad tadpole as it is in the Mexican axolotl according to the observations of Shufeldt (12). The mortality in this lot was very low, only 18 individuals having died at the time the record was made.

Lot B. — The tadpoles fed on wheat were, as a whole, considerably smaller than those fed on meat, and they were more uniform in size, the greatest number having a length of about 22 mm. By June 7, three individuals in this lot had begun their metamorphosis, and 38 individuals had died.

Lot C. — The smallest and least developed tadpoles were those that were fed on a mixed diet. The greatest extremes in size were also found in this lot, the body length varying from 10–21 mm. in different cases. In many individuals the hind legs were only just visible, and in the largest individuals they were poorly developed as compared with those of the individuals in other lots. The mortality in this lot was very great, 97 individuals having died by June 7.

Lot D. — The great majority of the tadpoles fed on the yolk of egg were intermediate in size between those fed on meat and those that had received a purely vegetable diet, the average length of these tadpoles being 22–24 mm. The individuals in this lot had developed much more rapidly than those in any of the other lots. By the seventh of June, 8 individuals had begun metamorphosis and many more were on the point of doing so. The mortality in this lot also was very great as 63 individuals had died.

The differences between the individuals in the various lots of Series II. were of the same character and as strongly marked as were those in Series I. The death rate in Series II. was practically the same as in Series I.; the fewest deaths occurring among the tadpoles that were fed on meat and on cereal; the greatest number among those that were nourished on a mixed diet and on the yolk of egg.

Although the tadpoles began to undergo their metamorphosis during the first week in June, the experiments were continued until the middle of July as there was a considerable variation in the rate of development among the tadpoles of the same lot. On July 13, all of the tadpoles still living were fixed in corrosive-

acetic, as they had reached a stage of development when it would be possible to ascertain the sex of each individual by means of a histological examination of the gonads.

In the corresponding lots of the two series of experiments there was a remarkable uniformity in the rate of development of the individuals. In both series the tadpoles fed on the yolk of egg underwent their metamorphosis much sooner than any of the others, the last one in Series II. completing its metamorphosis on July 11. These tadpoles were only of average size, and none of them ever reached the length attained by many of the tadpoles that were fed on meat. Lecithin, therefore, may cause a more rapid development, but it does not produce individuals of unusual size. The tadpoles fed on meat grew enormously but this increase in size was not accompanied by a more rapid development ; on the contrary, the development of these tadpoles seemed to be greatly retarded and some 50 of them had not begun metamorphosis by the middle of July. According to Yung, a purely vegetable diet is insufficient to transform a frog tadpole into a frog. Such a diet does not seem to be equally injurious to toad tadpoles, however, as comparatively few of the individuals that were fed entirely on wheat died during the course of the experiments, and only about 25 of them had not undergone metamorphosis by July 13.

As presumably the individuals that were fed on a mixed diet received the kind of food that is obtained by tadpoles living under natural conditions, it might be expected that these individuals would be larger and stronger than the others and that they would undergo metamorphosis more quickly than those receiving food that is only exceptionally, if ever, obtained by tadpoles in a state of nature. Much to my surprise the development of the individuals in Lot C lagged behind that of the tadpoles in the other lots, and large numbers of them died during the course of the experiments. On July 13, there were at least 100 tadpoles in Lot C that had not yet begun their metamorphosis.

The sex of all of the individuals used in the experiments was ascertained when possible. The results for Series I. are summarized in the following table.

TABLE II.

Character of Food Given.	Total Number of Individuals	Sex Not Ascertained.	Males	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	300	17	146	137	48.40	283
Wheat (Lot B).	300	38	119	143	54.58	262
Mixed food (Lot C).	300	108	103	89	46.35	192
Yolk of egg (Lot D).	200	49	55	96	63.57	151
Total.	1100	212	423	465	52.36	888

Table III. summarizes the results for Series II.

TABLE III.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	200	19	72	109	60.22	181
Wheat (Lot B).	200	16	76	108	58.69	184
Mixed food (Lot C).	200	43	86	71	45.22	157
Yolk of egg (Lot D).	200	74	56	70	55.55	1126
Total.	800	152	290	358	55.24	648

The first conclusion that can be drawn from the above tables is that abundant nutrition alone is not a decisive factor in sex determination in *Bufo*, as in three cases (Series I., Lot A, Lot C ; Series II., Lot C) more males than females were produced although all of the tadpoles had been well supplied with food during the entire course of the experiments.

As the tables show, the results of the two series of experiments in which the tadpoles were fed exclusively on meat are not in agreement.   In Lot A of Series I., only 48.4 per cent. of the individuals in which sex was ascertained were females ; while in the corresponding lot in Series II. there were many more females than males (20.44 per cent.).   This result does not support Yung's contention that an excess of nitrogenous food leads to the development of a greater proportion of females, and it seems to indicate that food of this character has no influence in determing sex in *Bufo*.   Again more rapid growth, as shown in the case of the tadpoles that were fed on the yolk of egg, cannot be considered as favoring the development of one sex any more than the other ; for although in both series there was an excess of females in Lot D, this excess varies considerably in the two series (8.02 per cent.)

and is not sufficiently great in either case to warrant the conclu-
sion that sex has been influenced by the rapid development due
to the character of the food.   The tables show also that a strictly
vegetable diet has seemingly no influence on sex determination
in *Bufo*.   The slight excess of females in Lot B of each series is
but little more than that which, according to my investigations,
is the normal excess for the species, and it is therefore well within
the limits of possible normal variation.   In both series the devel-
opment of the tadpoles that were nourished on a mixed diet (Lot
C) was, for some unknown reason, considerably retarded and the
individuals that completed metamorphosis were, as a rule, smaller
than those of any of the other lots.   Both series gave an excess
of males in Lot C.   This excess, however, is not great enough to
justify the assumption that a slow development tends to produce
a greater proportion of males, any more than the excess of females
among the tadpoles fed on the yolk of egg warrants the conclu-
sion that rapidity of growth favors the development of a greater
proportion of females.

The results of these experiments, therefore, seem to show that
the character of the food received by the tadpoles is not in itself
a decisive factor in determining sex in *Bufo*, although it has much
to do with the rate of development and with the size of the
individuals.

The results of the experiments as given in Tables I. and II.
are summarized in Table IV.

TABLE IV.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat.	500	36	218	246	53.01	464
Wheat.	500	54	195	251	56.27	446
Mixed food.	500	151	189	160	45.84	349
Yolk of egg.	400	123	111	166	59.92	277
Total.	1900	364	713	823	53.58	1536

Of the total of 1,536 individuals in which sex was ascertained,
823 or 53.58 per cent. were females.   The excess of females,
therefore, is but 1.7 per cent. more than the normal excess as
ascertained by the examination of the sex of 500 young toads

which had developed under natural conditions.   The number of females is greatest in the lot of tadpoles fed on the yolk of egg, being 8.1 per cent. above the normal ; and it is least in Lot C where it falls 5.9 per cent. below the normal.   These figures are, however, well within the limits of possible normal variation for the frog as determined by the investigations of Pflüger and Griesheim, and presumably, therefore they are also within the limits of normal variation in *Bufo*.

It has been suggested by Born, and emphasized by other investigators (Cuénot, Morgan (8)) that the results obtained in feeding experiments may possibly be influenced by the mortality that occurs during the course of the experiments, individuals of one sex dying more readily than those of the other.   During the course of my experiments from 30–150 individuals in each lot died before metamorphosis.   These individuals, as I have stated, were preserved and the sex ascertained when possible by means of sections.   From the records that were made it appears that tadpoles of one sex did not die in greater numbers than those of the other.   In the entire number of individuals that were examined the proportion of the sexes was practically the same ; in some lots the females died in greater numbers than the males, while in other lots the reverse was the case.   These results confirm Pflüger's contention that there is no relation whatever between mortality and sex among tadpoles reared under artificial conditions.

Taking into consideration the entire number of individuals used in the experiments, it is found that in the total of 1,900 tadpoles, 823 or 43.31 per cent. developed into females ; 713 or 37.52 per cent. became males ; leaving 364 or 19.15 per cent. in which the sex of the individuals was not ascertained.   If we assume, for the moment, that all of the individuals belonging to this 19.15 per cent. would have developed into females (although the investigation of the sex of the individuals that died during the course of the experiments does not warrant such an assumption), the number of females would then be increased to 1,187 or 62.47 per cent. of the whole number of individuals ; on the other hand, if all of the in dividuals in which the sex was not ascertained had developed into males, then the number of males would be 1,077

or 56.68 per cent. of the whole number of individuals. On neither of these assumptions is the proportion of the sexes in the 1,900 individuals changed sufficiently to justify the conclusion that the nutrition has any influence in the determination of sex in *Bufo*. The results of these experiments, taken as a whole or in part, seem to show that sex is not determined either by the quantity or by the quality of the food that the larvæ receive. This conclusion agrees essentially with that reached by Cuénot from the results of his investigations on frogs, moths and other forms, and by Schultze from his experiments on mice.

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THE FORMATION OF THE FIRST POLAR SPINDLE IN THE EGG OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

In a previous paper on " The Maturation and Fertilization of the Egg of *Bufo lentiginosus*" (King, 10), the formation of the first polar spindle and the subsequent divisions of the chromosomes were very incompletely described owing to a lack of material showing the details of these processes. During the spring of 1899, a large number of toads were collected soon after they had emerged from their hibernation, and from three of them sufficient material was obtained to give a more complete history of the late maturation processes. A short account of my study of this period in the development of the egg has already appeared (11); a detailed account is given in the present paper.

I. MATERIAL AND METHODS.

As soon as possible after the toads were captured they were killed by pithing and the body opened at once to ascertain the condition of the ovaries. In a great majority of cases the eggs were found free in the cœlomic cavity and were, therefore, of no use for the purpose intended, as previous investigations had shown that eggs which have broken through the wall of the ovary invariably contain a fully formed maturation spindle lying at the periphery near the center of the black hemisphere.

In several instances, all of the eggs were still attached to the walls of the ovaries when the toad was killed. In these cases some of the eggs were put at once into a dish of fresh spring water and the rest were left in the body of the female which was

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kept in a moist chamber. A few eggs from each of these series were then fixed at intervals of ten minutes for a period of several hours. By opening an egg under a dissecting lens after it has been taken from the fixing solution and put into 50 per cent. alcohol, one can tell definitely whether the late maturation processes have begun or not; for, if the nuclear membrane in still intact, the nucleus retains its rounded form and can be readily separated from the rest of the egg contents. If one hour after the toad is killed, an examination of freshly fixed eggs shows that the nucleus is still intact, the entire set of eggs can be discarded, as it has been found that further development does not take place unless the germinal vesicle breaks down previous to this time, although the eggs, whether kept in water or in the body of the female, show no signs of disintegration for many hours.

In one case the germinal vesicle was just breaking down when the eggs were first examined under the dissecting lens; in another set of eggs the germinal vesicle could no longer be dissected out half an hour after the toad was killed. These two lots of eggs gave overlapping series of stages which corresponded in every respect. A third set of eggs showed no signs of the germinal vesicle when first examined, and when sectioned showed maturation processes identical with those taking place in eggs which had been developing in water for several hours.

In all these three sets of eggs, the first polar body was given off in the normal position and apparently in the normal manner before the eggs showed any signs of disintegration. No difference was noticed in the development of eggs which had been put into water and those which had been left in the body of the toad. It does not seem, therefore, that such unusual conditions interfere at all with the late maturation processes provided these processes have started before the normal conditions are changed. No attempt was made to fertilize these eggs artificially, as it has never been found possible to fertilize either the eggs of *Bufo* or of *Rana* until they have received the thick jelly-like membrane which is secreted around them in the oviducts.

In all cases the eggs were fixed in corrosive-acetic and stained with a combination stain of borax carmine and Lyon's blue as described in a previous paper (King, 10).

II. The Disintegration of the Germinal Vesicle.

I have already given in detail a description of the early stages in the breaking down of the germinal vesicle, and as this new material confirms but adds nothing to that description, it will be necessary to give only a brief account of the changes in the egg directly preceding the formation of the spindle.

At the end of the hibernation period the germinal vesicle lies in the upper hemisphere of the egg. It is round in outline and contains a large number of nucleoli which usually form a ring enclosing the chromatin threads. A layer of granular substance staining differently from the cytoplasm, surrounds the lower pole of the germinal vesicle and extends half way up each side. This substance appears homogeneous at first and then becomes a compact, fibrous band of uniform thickness. I have called this band a "line of radiation," because, as soon as the nuclear membrane has disappeared in this region, the karyoplasm of the nucleus forms into coarse granules and a pronounced radiation extends up into the nuclear substance from the entire length of the fibrous band below. The karyoplasmic granules soon become smaller and more numerous and finally disappear entirely, while the radiation from below continues to increase and often extends nearly to the upper surface of the egg. The rays forming this radiation are very fine, and their outer ends run, apparently, into the coarse network which comes to fill the entire space formally occupied by the germinal vesicle. During these changes, the nucleoli have lost their power of staining and have begun to disintegrate.

When the nuclear membrane breaks down, twenty-four chromosomes, arranged in pairs, are scattered throughout the upper part of the nuclear space. The ends of each pair then unite to form a closed ring near which a small aster usually appears. The aster has no centrosome and its rays rarely touch the chromatin ring. At the next stage, when the radiation from below has reached its greatest extent, the asters and the chromatin rings entirely disappear. Later, when the radiation has begun to decrease, a large number of small round chromatin granules are found near or on the line of radiation which has been gradually shortening during this period. When the chromatin granules

first appear they stain very faintly, but they soon take the deep carmine stain characteristic of chromatin, and then fuse into several large, irregular clumps.

III. The Formation of the First Polar Spindle.

The line of radiation, shortly after the appearance of the chromatin granules, is shown in Fig. 1. It is a short, fibrous band with its ends, usually, though not invariably, slightly curved in towards the center of the egg. This structure, which is to become the first polar spindle, lies some distance below the surface of the egg in a small accumulation of granular substance formed, possibly, from the karyoplasm of the germinal vesicle. Its longitudinal axis may be either parallel or oblique to the surface of the egg, the latter position being the more common. Running out in every direction from the compact meshwork of fibers are numerous fine, thread-like rays which are longest and most numerous at the middle of the forming spindle where they extend out between the yolk spherules and seem to be continuous with the cytoplasmic network of the egg.

Collected near the middle of the spindle is a mass of small chromatin granules which are of uniform size and stain but faintly in comparison with the chromosomes of an earlier and of a later period. There is a very large number of these granules and it is quite impossible to count them satisfactorily; two other sections of the same egg each show as many granules as are shown in Fig. 1.

The nucleoli from the germinal vesicle appear at this period as irregular, yellowish green, refractive bodies which are scattered throughout the upper hemisphere of the egg, often lying quite close to the spindle. They disappear at different times in different eggs. Sometimes they have all been absorbed before the chromosomes have divided; sometimes they can still be found after the first polar body has been given off. I have never found any traces of them, however, after the spermatozoön has entered the egg.

Not more than fifteen minutes after the stage of Fig. 1, the chromatin granules begin to fuse into irregular-shaped clumps. The number and size of these clumps vary greatly in different eggs, in some cases there are but four or five of them, in others

at least twenty.   Owing, probably, to their greater volume, these larger masses always stain much more intensely than do the small granules.   Meanwhile the spindle has lost its uniform diameter and has become much thicker in the middle where the meshwork of fibers appears more distinct and more regular.   The spindle soon becomes barrel-shaped and its fibers are quite clearly defined in the middle region but not at the poles (Fig. 2).   The radiation from the spindle disappears entirely except at the poles where it forms distinct asters; some of the rays are very long and cross each other at the equator of the spindle.   During its migration towards the upper pole of the egg the spindle shortens somewhat and gradually becomes more slender and pointed, a phenomenon seen by Van Name (17) in the eggs of Planarians, by Korschelt (12) in *Ophryotrocha*, by Griffin (8) in *Thalassema*, and by Boveri (1) in *Ascaris*.

At no stage in the formation of the spindle or in its later history can any centrosome be found in the polar asters.   As the spindle becomes more pointed, the rays converge more sharply at the poles, but even when the radial systems are best developed (Figs. 2, 3), the rays appear to run into each other in the center of each aster and there is not the slightest trace of any kind of a central body.   Carnoy and Lebrun (2) in their study of the batrachian egg, Eismond (5) in his work on *Siredon* and *Triton*, Fick (6) in studying the maturation of the Axolotl egg, and *Sobotta* (15, 16) in working on the egg of the mouse and of *Amphioxus*, have all failed to find a centrosome in the asters of the polar spindles.   If such a structure is normal in these eggs and also in the egg of *Bufo lentiginosus*, methods of fixation and staining which have so clearly demonstrated its presence in other eggs are totally inadequate in these cases to show the slightest trace of it.

At the stage of Fig. 3, the small chromatin granules have entirely disappeared.   Whether they have all gone into the large chromatin clumps or whether some have been absorbed by the cytoplasm cannot be determined.   At this time the number of large chromatin masses still varies slightly in different eggs; in some cases there are nine such clumps of chromatin, in others at least fifteen.   These chromatin masses are, for the most part, scattered irregularly along the spindle fibers, occasionally, however, one or more of them can be seen entirely outside of the

spindle (Fig. 4, *CM*). Isolated masses of chromatin are some-
times found near the spindle at a much later period when the
chromosomes are at the equator preparing to divide. They have
entirely disappeared by the time the first polar body is given off,
possibly serving as food for the cytoplasm as suggested by Gar-
diner (7).

During the next half-hour, the irregular chromatin masses
change into chromosomes with a definite shape. The change
does not take place at the same time in all of the chromatin
clumps; in fact, until the chromosomes are arranged at the equa-
tor of the spindle ready to divide, they may be found in several
different stages of development on the same spindle. Twelve
chromosomes, one-half the number characteristic of the somatic
cells of this species differentiate from the chromatin masses. The
chromosomes are scattered over the entire spindle and are at first
somewhat triangular in shape (Fig. 3), later they become rod-
shaped structures which may lie with their long axis parallel,
oblique, or even at right angles to the longitudinal axis of the
spindle (Fig. 4). Sooner or later, however, the long axis of each
chromosome comes to lie parallel with the spindle fibers and the
chromosomes then have a rounded knob in the middle region
and frequently also a smaller knob at each end (Figs. 4, 5).
Later the middle knob becomes more prominent and the end
knobs disappear (Fig. 5).

At the stage of Figs. 2–3 the asters at the spindle poles reach
their greatest development. There are many long rays from
each aster which run nearly parallel with the spindle fibers and
cross each other at the equator of the spindle, and fewer and
much shorter rays going out in other directions. Soon after this
time the asters begin to degenerate. The shorter rays disappear
first and by the time the spindle has reached the periphery of the
egg there is not a trace of the radiation left. The spindle fibers
then converge at the poles which are surrounded by a small ac-
cumulation of granular substance probably formed from the dis-
integrated rays (Fig. 7).

There is often a marked difference in the size of the chromo-
somes on the same spindle even when they are of exactly the
same shape. One or two of the chromosomes may extend over
one-third the length of the spindle, the others being not more

than one-half as large (Fig. 5). This difference is not found at
a later period ; for, when the chromosomes are arranged at the
equator of the spindle ready to divide, they are considerably
smaller than the chromosomes of an earlier period and are all,
apparently, of the same size.

While the chromosomes are being arranged at the equator of
the spindle they undergo further changes in form. The polar
arms shorten considerably, while the thick knob at the middle
increases in size and gradually spreads out laterally, thus forming
two wing-like projections on the chromosomes (Figs. 6, 7). In
proportion as the lateral wings grow larger the polar arms of the
chromosomes become shorter and thinner, so that there can be
no question but that this lateral growth takes place at the ex-
pense of the rest of the chromosome. In a dorsal view, the
wings appear to be spread out flat on the spindle and the chro-
mosome has the appearance of a cross in which the polar arms
are somewhat longer than the equatorial arms (Fig. 6). In a
lateral view, however, the wings are seen to be raised up from
the spindle while the polar arms are extended along the spindle
fibers. Carnoy and Lebrun (2) have applied the term "oiselet"
to this stage in the development of the chromosome. The typ-
ical oiselet stage is followed by one in which the body of the
"bird" gradually disappears while the wings constantly increase
in size (Fig. 7). Very soon, all that is left of the original polar
arms is a slight projection on each side of the angle formed by
the meeting of the two wings (Fig. 8). In the succeeding stage
every trace of the polar arms has disappeared and there are
twelve broad V-shaped chromosomes arranged at the equatorial
plate with the angle of the V turned in towards the center of the
spindle (Fig. 9). Usually, before this last stage is reached, the
spindle has come to lie close to the surface of the egg and nearly
radial in position. This is by no means invariably the case, how-
ever, as sometimes the spindle is still some distance below the
surface of the egg when the chromosomes have divided in prepa-
ration for the first maturation division.

Fig. 6 shows part of a section of an egg fixed as soon as pos-
sible after the toad was killed. The spindle lies at the periphery
of the egg and the chromosomes, with well-developed lateral
wings, are at the equator. That this egg and others from the

same series are normal cannot be questioned.   They show phe-
nomena exactly similar to those seen in eggs that have been
developing in water for some three hours, and leave no doubt but
that the earlier processes described above are normal in spite of
the unusual conditions to which many of the eggs were subjected·

Four V-shaped chromosomes in which all traces of the polar
arms have disappeared are shown in Fig. 9.   The arms of the
V's are broad flat plates which form a sharp acute angle with
each other.   There is, in this case, no sign of a splitting in any
of the chromosomes which are all of the same size and shape
and arranged at the equatorial plate with the angle of the V
turned in towards the center of the spindle, a characteristic ar-
rangement of the chromosomes at this period.   An equatorial
section of a spindle in the same stage as Fig. 9, is seen in Fig.
10, where all twelve chromosomes are present.   In this egg
there are also found near the spindle a number of nucleoli which
are in the process of disintegration.

Usually the first indication of any division of the chromosomes
is seen at the stage of Fig. 11 when the polar arms have entirely
disappeared and the chromosomes are broad V-shaped structures.
At this time the ends of the V's often show a deep indentation
(Fig. 11) indicating the longitudinal splitting of the chromosomes·
Occasionally I have found the first division coming in at an
earlier period before the entire disappearance of the polar arms.
Such a division is seen in the chromosome at the left in Fig. 7.
In all such cases the splitting is confined entirely to the lateral
wings and never extends into the polar arms.

In the egg from which Fig. 12 was drawn, there are twenty-
four V-shaped chromosomes which are similar to the twelve
chromosomes in Fig. 10 in every respect except that they are
much narrower.   They have been produced, I believe, by a
longitudinal division of the broad V-shaped chromosomes found
at an earlier period.   In some of the chromosomes shown in
Fig. 12, the division for the second polar mitosis is seen.   This
second division of the chromosomes is not visible, at this stage,
except in equatorial sections of the spindle.   In longitudinal sec-
tions of the spindle the chromosomes always appear to be ar-
ranged in tetrad groups, one of which may be seen in Fig. 12.
Such a group is, in reality, a pair of V-shaped chromosomes with

the angle of each V turned in towards the center of the spindle, the four ends of a pair of chromosomes projecting from the spindle give the appearance of a typical tetrad. The maturation divisions of the chromosomes are represented diagrammatically by text-figures 1–5.

In my previous paper, three sections from one egg (Figs. 25, 26, 27) were given in which the fully formed spindle lay some

1   **2**   **3**   **4**   **5**
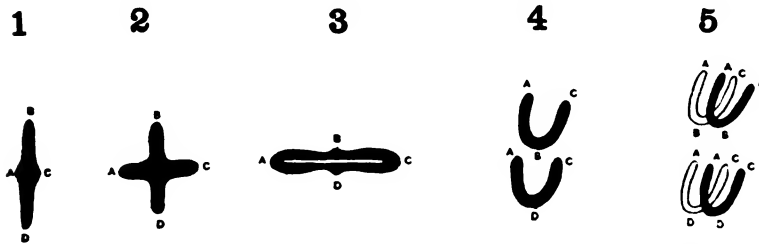
Diagrams of the maturation divisions of the chromosomes in the egg of Bufo lentiginosus.

distance below the surface of the egg and the chromosomes were in the form of closed rings which were split longitudinally. This egg was undoubtedly abnormal and led to the wrong inference that these chromatin rings were identical with those found in the germinal vesicle just previous to its disintegration. If the split V-shaped chromosomes of Fig. 11 were to be spread out in the form of a ring and the second maturation division to take place before the halves of the ring separated, then exactly the same effect would be produced as previously illustrated in Figs. 25–27. I can only interpret the ring-shaped chromosomes in this abnormal egg — the one abnormality I have found in many hundreds of eggs sectioned — as due to a delay in the separation of the parts after the two divisions of the chromosomes had taken place.

According to Carnoy and Lebrun (2, 3) who have published a series of memoirs dealing with the development of the germinal vesicle and the formation of the polar bodies in the eggs of various Batrachia, the chromatin filaments in the egg of Salamander, *Alytes, Triton, Bufo* and *Rana* arise from repeated resolutions of the nucleoli in the germinal vesicle. As my own work on *Bufo* began with the fully formed egg taken from the animal just before the beginning of the hibernation period, I have not yet seen this

resolution of the nucleoli into chromatin threads. In all the
eggs that I have examined in which the germinal vesicle was in-
tact, the chromatin was always in the form of distinct chromo-
somes. These chromosomes had no connection whatever with
the large round nucleoli which, with the combination stain used,
always stain a deep blue while the chromatin invariably takes
the carmine. I have frequently noticed, however, that many of
the chromosomes end in small granules which take the same
stain as the chromatin and that there are a number of simi-
lar granules scattered throughout the nucleus. Recent work on
various forms has shown that unquestionably the term nucleolus
has been applied to many different kinds of structures in the
germinal vesicle. As a general term used to cover any definite
structures in the germinal vesicle other than chromosomes, linin
and karyoplasm, it may, perhaps, be fitly applied both to the
large rounded structures (which I consider the only true nucleoli
in the germinal vesicle) and to the smaller granules which stain
like chromatin and which I believe to be chromatin that is not
used for the chromosomes. The structures which, in my
opinion, are the true nucleoli have nothing to do with the forma-
tion of the chromosomes for the first polar spindle, as they are
never connected with the chromosomes in any way and can be
traced step by step until they are absorbed by the cytoplasm of
the egg after the spindle is completely formed.

Many of Carnoy and Lebrun's illustrations of the forma-
tion of the first polar spindle in the egg of *Bufo vulgaris* are
strikingly like my own, but we differ somewhat in our interpre-
tation of them. According to their view, when the germinal
vesicle in the egg of *Bufo vulgaris* migrates towards the upper
pole, and before the nuclear membrane disappears, the paired
chromatin filaments (which are exactly like those I find in *Bufo
lentiginosus* during the same period) break up into small granules
which cannot be distinguished from the granules of karyoplasm.
*All the nucleoli suffer the same fate as the chromosomes excepting
about ten which remain to form the chromosomes of the first polar
spindle.* The karyoplasm meanwhile, forms a pronounced radia-
tion from the " plage fusoriale " at the lower pole of the germinal
vesicle. " Les nucléoles prédestinés montent le long des fila-

ments" and are carried to the "plage fusoriale" where they either become vacuolated in the center and form a ring, or else they fuse into one large mass and later regain their individuality. When the spindle is first formed, the chromosomes are very irregular in shape and there are distinct asters at the spindle poles which never contain a centrosome.

In the egg of *Bufo lentiginosus*, I have traced the chromosomes of the germinal vesicle up to the stage where the ends of a pair of chromosomes unite to form a closed ring. After this time, although I have had an abundance of material and have searched very carefully through every section of the germinal vesicle in a large number of eggs, I have been unable to find any trace of the chromatin. There is, I believe, no doubt but that the chromatin rings break up into minute granules which may, possibly, be carried by the karyoplasmic radiation to the lower pole of the germinal vesicle where they later form the chromosomes of the first polar spindle. I have never seen anything in this egg, however, that would indicate that some of the *nucleoli* are destined to form the chromosomes of the first polar spindle. A large number of nucleoli are always present throughout the early stages of maturation and they all appear to be undergoing the same processes of disintegration. Carnoy and Lebrun might consider the large irregular masses shown in Fig. 2 to be nucleoli in the general sense in which they seem to use the word, but these masses have been formed by the fusion of smaller chromatin granules (Fig. 1) and are in no way connected with the true nucleoli of the germinal vesicle.

Carnoy and Lebrun have followed the details of the formation of the first polar spindle and the later changes of the chromosomes much more carefully in the egg of *Triton* than in any of the other amphibian eggs they have studied. Their account of this form agrees substantially with that of *Bufo vulgaris* as regards the breaking down of the germinal vesicle, with the important exception that in *Triton*, *all* of the nucleoli are absorbed by the cytoplasm, none of them are reserved, as in *Bufo Vulgaris*, to form the chromosomes of the first polar spindle. The chromatin threads which were resolved from the nucleoli at an earlier period, break up into very small granules when the membrane

of the germinal vesicle disappears. The twelve chromosomes which later arise *from a coalescence of the chromatin granules* are at first very irregular in shape and they are scattered all along the spindle fibers; subsequently they undergo a double longitudinal division in preparation for the giving off of the polar bodies. Any chromatin not used for the chromosomes is absorbed by the cytoplasm.

Although there are always twelve chromosomes on the first polar spindle in the egg of *Triton*, Carnoy and Lebrun find only 8–10 chromosomes in the equatorial plate of the first polar spindle in the egg of *Bufo vulgaris*, and but 4–5 chromosomes at each pole just previous to the giving off of the first polar body. The failure of these investigators to find the definite number of chromosomes that must be present unless the egg of *Bufo vulgaris* is a marked exception to the rule that the number of chromosomes is constant for a given species, may possibly be accounted for on the supposition that some of the chromosomes were lost when the eggs were sectioned or that the sections of the egg were made so thick that some of the chromosomes were not visible.

In a more recent paper, Lebrun (13) gives the results of a reexamination of the maturation processes in the egg of *Triton*. He states that the double longitudinal division of the chromosomes does not take place in the complicated manner previously described by Carnoy and Lebrun, but according to the scheme represented by my text-figures 1–5. The late maturation changes in the egg of *Triton* are, therefore, strikingly similar to those I have found taking place in the egg of *Bufo lentiginosus*. Lebrun still believes that in the eggs of *Rana temporaria* and of *Bufo vulgaris* a certain number of the nucleoli are reserved to form the chromosomes of the first maturation spindle. A reëxamination of the maturation stages in the eggs of these amphibians would probably show that in these forms also the chromosomes are derived from fused masses of chromatin granules and that they have no connection whatever with the true nucleoli.

I have examined a large number of the eggs of *Bufo* at the stages of Figs. 3–4 and I can see no reason for believing with Carnoy and Lebrun that a division of the chromosomes Takes

place at this time. During this period the chromosomes are exceedingly varied in size and shape. If the chromosome is oblong, it may have either its long or its short axis parallel with the longitudinal axis of the spindle; if the chromosome is pyramidal in shape, either the base or the apex of the pyramid may rest on the spindle fibers. I regard all of the changes in the shape of the chromosomes up to the stage of Fig. 7 as due solely to a rearrangement of the chromatin material preparatory to the later divisions. The first indication of any division of the chromosomes is the longitudinal splitting of the lateral wings which in some few cases can be found before the disappearance of the polar arms (Fig. 7). The apparent separation of the lateral wings at X, Fig. 11, I consider to be due to the fact that the angle of the V-shaped chromosome was cut off in sectioning. It very frequently happens that portions of one or of several chromosomes on a spindle are removed in this way. Sometimes, as in Fig. 4, the median knob of a chromosome is lacking; sometimes, the lateral wings have been removed (Figs. 6, 7). In rare instances the cut off portion of the chromosome will be found in the next section of the egg; but as the chromosomes are quite small a careful examination of the following sections often fails to disclose the missing part.

As found to be the case in many eggs besides that of *Bufo*, for example in *Cerebratulus* (Coe), *Polychærus caudatus* (Gardiner), *Thalassema* and *Zirphæa* (Griffin), and *Triton* (Carnoy and Lebrun), all the chromatin of the germinal vesicle does not go to form the chromosomes of the first polar spindle, some of it is thrown out into the cytoplasm where it degenerates and sooner or later completely disappears. Even in the segmentation stages of the egg of *Ascaris*, Boveri (1) found that some of the chromatin is thrown out of the nucleus and absorbed by the cytoplasm. In all these cases there is obviously a mass reduction of the chromatin in preparation for the succeeding division of the cell. It may be, as suggested by Gardiner, that " there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm." This theory would explain the facts as we now know them, but it cannot be proved until some stain can be found to differentiate the two sorts from each other.

Carnoy and Lebrun find a double division of the chromosomes in the egg of *Triton*, and they state that there is no reason why a longitudinal division of the chromosomes should not be a reduction division in the Weismann sense, in that it may separate the chromosome into two parts each containing different kinds of granules : it is certainly true if we admit a difference in the properties of the elementary granules. As all of the chromatin granules do not go into the chromosomes of the first polar spindle, there is a process of selection in the formation of the chromosomes and their subsequent division would be a permanent source of variation for the descendents.

The chromosomes of the first polar spindle in the egg of *Bufo lentiginosus* are at first exceedingly varied in shape ; they may be round, triangular, or oblong. At this time it is obvious that they have no definite longitudinal axis. At the stage of Fig. 5 the chromosomes have elongated and lie parallel with the longitudinal axis of the spindle. When the wings have formed, there is a stage when the arms of the chromosomes are all approximately of the same length (Fig. 6). Is there a definite longitudinal axis at this time ? If the part of the chromosome resting upon the spindle fibers is considered to be the longitudinal axis, then later this same axis is not only shorter than the transverse axis, but it practically disappears at the stage of Fig. 9. If shown Fig. 9 without the preceding figures, no one, I am sure would call the thickness of the chromosome at the angle of the V the longitudinal axis of the chromosome, and the division indicated in Fig. 11 would unhesitatingly be called a longitudinal division. If one arbitrarily states that the polar arms of the chromosomes in Fig. 5 form the true longitudinal axis, not only in this particular stage, but until division is completed, then the splitting seen in Fig. 11 is a transverse division, as is also the second division which takes place in the same direction. On the other hand, if the longer axis of the chromosome at the time when division occurs is considered to be the true longitudinal axis, then there is a double longitudinal division of the chromosomes and the egg of *Bufo* is thus brought into line with other amphibian eggs that have been studied. It would seem, as suggested by Sebaschnikoff (14), that the distinction between transverse

and longitudinal divisions of the chromosomes is not as impor-
tant as many investigators have claimed : the division of the
chromatin substance would appear to be the important thing, the
manner of its achievement quite secondary, as Hertwig (9) has
maintained.

There is, however, the following possibility to be considered.
When the germinal vesicle breaks down, all of the chromo-
somes are arranged in pairs, in some cases the ends of a pair
of chromosomes have united to form a closed ring.   Very soon
after this stage the chromosomes break up into granules and
all traces of the chromatin substance is lost until innumer-
able chromatin granules appear in connection with the first polar
spindle.   It is conceivable that all of the chromatin granules be-
longing to a pair of chromosomes have remained united during
this period of the apparent disintegration of the chromosomes,
although I have not been able as yet to demonstrate such a
union.   If such is the case, then the chromosomes of the first
polar spindle are bivalent structures, each being composed of the
two chromosomes that had become paired at an earlier period of
development.   On this assumption it is probable that the knob-
like thickening in the middle of the chromosomes, shown in Figs.
4 and 5, is caused by the fusion of the ends of the two chro-
mosomes.   In text-figure 1, ABC and ACD would represent the
two chromosomes united at AC.   The subsequent changes in
the shape of the chromosomes serve merely to again elongate
the original chromosomes (Text-fig. 3) which are finally separated
by the division through AC.   The first maturation division,
therefore, is a reduction division and the second division only is a
longitudinal one.   It certainly cannot be mere chance that at the
time of the breaking down of the germinal vesicle, the chro-
mosomes should invariably become arranged in pairs.   In light
of the most recent investigations on spermatogenesis and ovo-
genesis it would seem as if the above explanation must be the
true one for the maturation divisions in the egg of *Bufo*, although
at present I am not able to prove it.   I hope that the work I am
doing on the spermatogenesis of this amphibian will throw some
light on the maturation divisions in the egg.

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All figures were drawn with the aid of a camera lucida under a Zeiss Apoc. 2 mm., Oc. 4.

1. An early stage in the formation of the first polar spindle before the chromatin granules have fused into large masses.

2. A stage about one-half hour later than Fig. 1. The spindle has become barrel-shaped and the chromatin granules are fusing into large masses to form the chromosomes.

3. Twelve irregularly shaped chromosomes have differentiated from the chromatin masses and lie scattered along the spindle.

4. Spindle parallel to the surface of the egg. The chromosomes have elongated and many of them show a median knob. *C. M.*, chromatin mass outside the spindle.

5. About the same stage as Fig. 4. Chromosomes of very different sizes are found on the spindle.

6. Typical "oiselet" stage.

7. Chromosomes in various stages of development on the same spindle. In some of the chromosomes the splitting for the first maturation division can be seen while the polar arms are still present.

8. Section showing the growth of the lateral arms of the chromosomes at the expense of the polar arms.

9. The V-shaped chromosomes after the disappearance of the polar arms.

10. An equatorial section of a spindle at the stage of Fig. 9. All twelve chromosomes are present.

11. Equatorial section. The notched ends of some of the chromosomes indicate the direction of the first maturation division.

12. Equatorial section. The first maturation division is completed and the second maturation division is indicated in some cases.
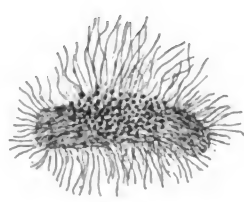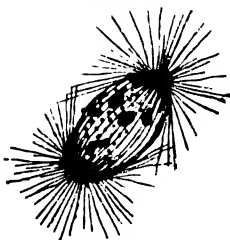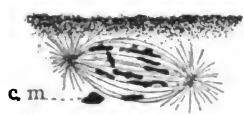
PLATE I

THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

HELEN DEAN KING.

THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

HELEN DEAN KING.

A study of the mode of development of the notochord in the common toad, *Bufo lentiginosus*, and of the frog, *Rana palustris*, has brought to light certain points that have a bearing on the formation of the same structure in related groups. A vast amount of work has already been done along this line, yet a wide difference of opinion exists among embryologists regarding the origin of the notochord in the Amphibia. It is hoped that the results recorded in the present paper may help to clear up this question.

The material used was fixed in corrosive-acetic (5° glacial acetic acid), and the sections were stained on the slide with a mixture of borax-carmine and Lyon's blue as described in a previous paper (King, 11). This stain gives particularly good results when it is used on freshly preserved material, as then all of the nuclei become dark red, the ectoderm and mesoderm appear dark blue, while the yolk cells take but a pale blue tint and, therefore, are easily distinguished from the other cells. This sharp definition of the tissues was of great assistance, particularly in the study of the sections of *Bufo*. All of the drawings given in the present paper were outlined with the aid of a camera lucida.

BUFO LENTIGINOSUS.

When the circular blastopore is closing in, the mesoderm, already differentiated from the other tissues, forms a continuous sheet of small, angular, slightly pigmented cells across the dorsal wall of the archenteron. In the middle and also in the anterior part of the embryo, the mesoderm is entirely separated from the ectoderm above as well as from the endoderm beneath it. In the region just in front of the blastopore, the mesoderm is also

distinct from the ectoderm, but it is united for some distance with
the cells forming the dorsal wall of the archenteron. At this stage
of development there is first noticed, in the middle of the em-
bryo, a pronounced thickening of the mid-dorsal mesoderm (Fig.
1, *N*), which extends only over a few sections at first and is con-
tinuous with the lateral mesoderm on either side.   When the
blastopore is nearly closed, the thickened portion of the mesoderm
is cut off from the lateral mesoderm to form the notochord, the
line of separation coming in at about the points marked *XX* in
Fig. 1.   As the embryo elongates, the forward extension of the
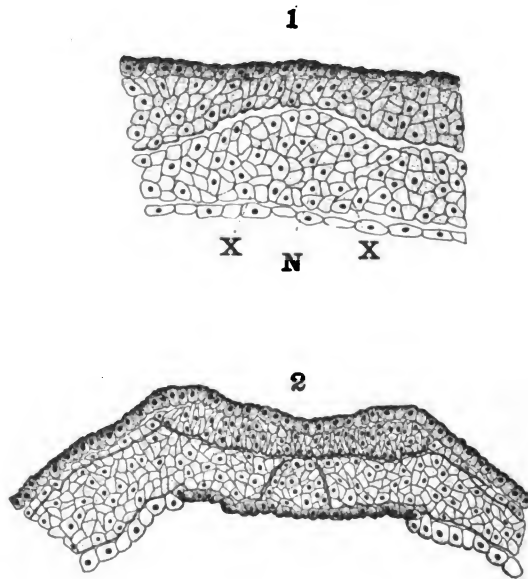
FIG. 1.   Part of a medium sagittal section through an egg of *Bufo lentiginosus*
in which the blastopore has begun to close.   *N*, thickening of mid-dorsal mesoderm
which is to be cut off at the points *XX* to form the notochord.

FIG. 2.   Part of transverse section through the posterior region of an embryo in
which the medullary plate has appeared.

notochord always takes place in this same way, *i. e.*, by the cut-
ting off, laterally, of a portion of the mesodermal layer in the
mid-dorsal region so that, from the beginning, the notochord is
entirely separated from the ectoderm and also from the endoderm.
These observations confirm the statement made in a previous
paper (King, 12) that "the anterior part of the notochord is cer-
tainly mesodermal in origin."

Transverse sections through an embryo in which the medullary plate has just appeared show that, in the anterior region, the notochord is composed of a rounded mass of cells cut off entirely from the surrounding tissues, and appearing much as in Fig. 7. In the posterior region, there is, as yet, no trace of a notochord, and an unbroken layer of cells extends across the dorsal surface of the archenteron, as here the mesoderm is still
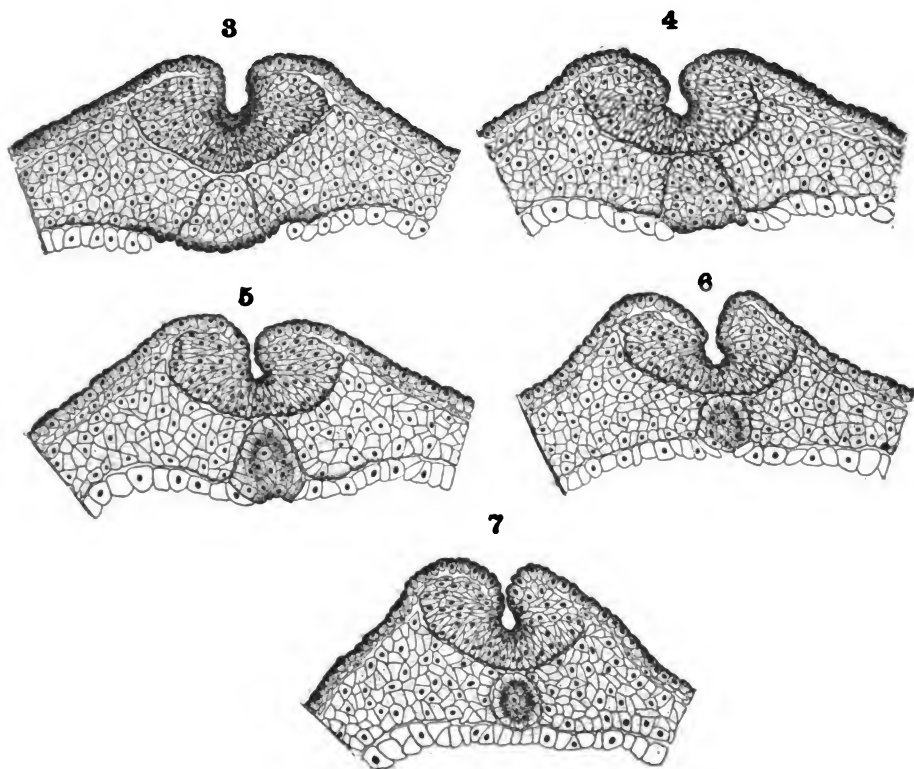
FIGS. 3–7. Serial sections from the posterior to the middle region of an embryo of *Bufo lentiginosus* in which the medullary folds are closing.

united with the endoderm as in the earlier stages. In a section made a short distance behind the middle of the embryo (Fig. 2), the notochord appears as a triangular shaped chord of cells, entirely distinct from the mesodermal layer on either side, but closely connected with the cells forming the mid-dorsal wall of the archenteron. In this part of the embryo, as well as more

posteriorly, the archenteron is surrounded on its ventral, lateral and lateral-dorsal surfaces by large, rounded. faintly staining yolk cells which contain very little, if any, pigment ; the mid-dorsal wall, on the contrary, is formed of a single layer of much smaller, rectangular cells which are very heavily pigmented on the side bordering the archenteron. This layer of cells, which I shall call " the dorsal plate," is broadest in the posterior part of the embryo, where, in transverse sections, it appears as a nearly straight line of cells covering about two-thirds of the mid-dorsal surface of the archenteron. More anteriorly the dorsal plate gradually becomes narrower, until it finally disappears completely in the middle of the embryo. The archenteron in front of this region is entirely surrounded by large yolk cells.

The outer cells of the dorsal plate, instead of grading into the yolk cells as one might expect, are found to be directly continuous with the lower layer of mesoderm. There is, therefore, in this region an abrupt change from the small, deeply pigmented cells of the dorsal plate to the large yolk cells which form the lateral and ventral walls of the archenteron. At no stage in the development of the embryo have I ever found any transitional stages between these two different kinds of cells. The cells of the dorsal plate resemble, in all respects, the cells forming the outer surface of the embryo, being of the same size and shape and containing about the same amount of pigment. From the results which I obtained in my study of the gastrulation of the egg of this species (King, 12), it seems highly probable that the cells composing the dorsal plate were invaginated from the surface ot the egg during the formation of the blastopore, and, consequently, they have had a very different origin from the cells forming the lateral and ventral walls of the archenteron which are all derived from the yolk portion of the egg.

When the medullary folds are closing, the mesoderm in the posterior region is still connected, for a short distance, with the cells forming the dorsal wall of the archenteron, and the notochord has not yet extended into this portion of the embryo. Fig. 3 shows a portion of the section through the region where the notochord has just been cut off from the mesoderm. This section corresponds in its position in the embryo with the position

of the section of the earlier embryo shown in Fig. 2. The notochord is triangular in shape and is closely connected with the layer of cells forming the mid-dorsal wall of the archenteron. The portion of the dorsal plate directly under the notochord is cut off on either side from the rest of the layer, and to it one can, perhaps, fitly apply the term " chorda-endoderm," since it is destined to become a part of the notochord. At this stage of development, the dorsal plate is much narrower in the posterior region of the embryo than it was before the medullary folds formed (Fig. 2), and it is again found to be directly connected with the lower layer of mesoderm and not with the yolk cells forming the lateral walls of the archenteron.

In Fig. 4, a portion of a section slightly anterior to that shown in Fig. 3, the chorda-endoderm is seen to be the only portion of the dorsal plate bordering the archenteron. The other cells of the dorsal plate have united with the mesoderm, and can only be distinguished from it on account of their position and the fact that they contain somewhat more pigment. The entire dorsal wall of the archenteron, excepting the part formed by the chorda-endoderm, is here composed of large, rounded yolk cells which are evidently growing up from both sides, and thus shutting off all of the cells of the dorsal plate from bordering the archenteric cavity. More anteriorly, as shown in Fig. 5, the yolk cells of the upper wall of the archenteron are still closer together in the middle lines. In this part of the embryo the cells of the chorda-endoderm no longer form a nearly straight line at the lower edge of the notochord, but they have become an integral part of it, and most of their pigment is collected in the form of a pronounced ring around the center of the notochord.

Near the middle of the embryo (Fig. 6), the yolk cells have almost met under the notochord, which is smaller and more rounded than it is in the posterior part of the embryo. A section more anteriorly still (Fig. 7) shows that the yolk cells from the two sides of the archenteron have come together in the middle line under the notochord. As a result, the dorsal wall of the archenteron is composed entirely of a single layer of large yolk cells, and the cylindrical notochord above it is cut off entirely

from the surrounding tissues. In the head region, the relation of the tissues is practically the same as that shown in Fig. 7.

When the medullary folds have closed, there is found in the posterior region of the embryo a much narrower dorsal plate than that shown in Fig. 3, as more of the cells have been covered over by the upward growth of the yolk cells from the sides of the archenteric cavity. Anteriorly the dorsal plate grows narrower very rapidly and some distance back of the middle of the embryo the yolk cells have already come to surround the entire archenteron. By the time that the optic bulbs have formed, there is no longer any dorsal plate in the mid-dorsal wall of the archenteron and the notochord has no connection with any of the surrounding tissues.

These results show that the anterior part of the notochord in the embryo of *Bufo lentiginosus* is entirely mesodermal in origin; in the posterior part of the embryo, the greater part of the notochord is also derived from the mesoderm, but there is added to it a single layer of chorda-endoderm from the mid-dorsal wall of the archenteron. Back of the middle region of the embryo, the yolk cells grow up from the lateral walls of the archenteron and unite under the notochord, the cells of the dorsal plate thus cut off from bordering the archenteron, either unite with the notochord or are incorporated into the splanchnic mesoderm.

RANA PALUSTRIS.

In the frog, *Rana palustris*, the notochord is formed at about the same stage of development that it is in *Bufo*, namely, near the end of gastrulation when the blastopore is closing in. As in the embryo of *Bufo*, the notochord first appears in the middle region as a rounded chord of cells cut off from the mid-dorsal mesoderm, and it is separated entirely from the ectoderm and also from the endoderm beneath which forms the dorsal wall of ʃhe archenteron. At this stage in the development of the egg, the mesoderm in front of the region where the notochord has been cut off forms a solid layer of cells extending across the dorsal wall of the archenteron and entirely separated from it ; the mesoderm back of the notochord also extends in an unbroken sheet across the mid-dorsal region, but in this part of the egg meso-

derm and endoderm are connected for a considerable distance on either side of the middle line.

In the posterior part of the embryo the cells forming the dorsal wall of the archenteron do not differ in size, shape, or in power of staining from the mesodermal cells above them, and at the sides of the archenteron they grade into the larger yolk cells forming the ventral and lateral walls.   There is a comparatively narrow region in the mid-dorsal wall where the edges of the cells bordering the archenteric cavity are quite heavily pigmented ; but the other cells of the dorsal wall contain about the same amount of pigment as do the mesoderm cells above them, and there is no definite dorsal plate of small, deeply pigmented, rectangular cells as in the toad embryo.   I can find no evidence that any of the cells of the dorsal wall of the archenteron ever form a permanent union with the mesoderm.

When the medullary folds are beginning to form, the notochord has extended into the posterior region of the embryo and appears as in Fig. 8.   It is a more rounded structure than is the notochord of the toad embryo at a corresponding stage of development (Fig. 3), yet it, too, is closely connected with the endodermal layer of cells forming the mid-dorsal wall of the archenteron.   As shown in Fig. 8, the lateral mesoderm and the endoderm of the archenteric wall are connected for some distance on either side of the notochord.   The cells of both of these tissues have the same general characteristics, and there is no sharp distinction between them as in the embryo of *Bufo*.   As all of the cells in the dorsal part of the embryo have the same power of staining, it is not easy to follow the changes that take place, even with an abundance of material for study.   Not until I had made camera drawings of a complete series of sections through the posterior region of an unusually favorable embryo was I able to tell with certainty how the notochord is formed.   Four of these drawings (from the same embryo as Fig. 8) are reproduced in Figs. 9–12.   For the sake of clearness only the dorsal wall of the archenteron and the notochord are shown.   In all of the sections the mesoderm is entirely cut off from the notochord, and also from the endoderm beneath it.

A short distance in front of the region shown in Fig. 8, almost

all of the pigment in the mid-dorsal wall of the archenteron is
found to be massed in the outer edges of a very few cells which
are entirely cut off from the other cells of the archenteric wall
and are attached to the lower surface of the notochord (Fig. 9).
These few cells are undoubtedly comparable to the layer of
chorda-endoderm found in the mid-dorsal wall of the archenteron
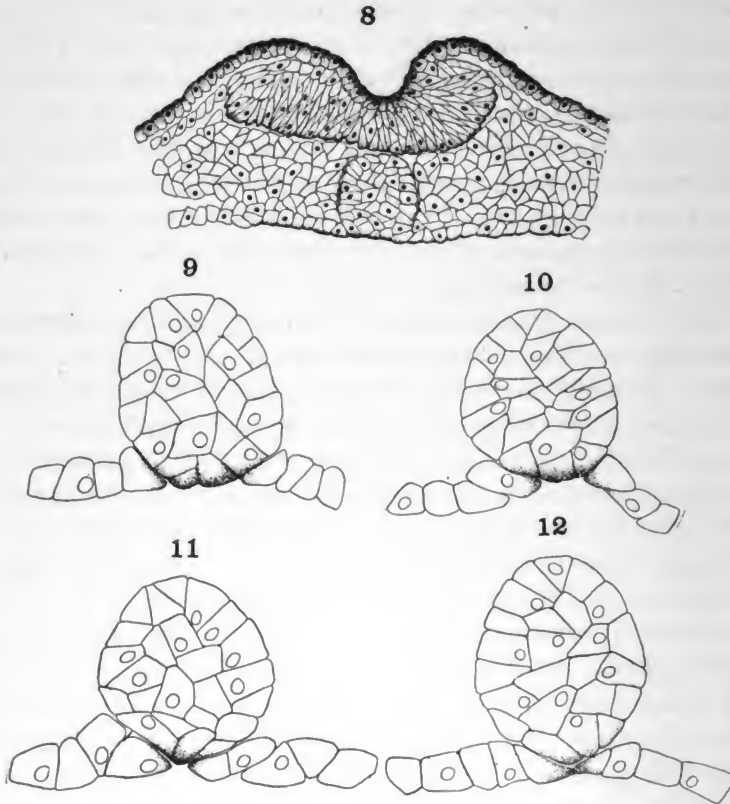
FIGS. 8–12.   Serial sections from the posterior to the middle region of an embryo
of *Rana palustris* in which the medullary folds are closing.

in the toad embryo, and, therefore, the same term may fitly be
applied to them.   More anteriorly (Fig. 10) there is a noticeable
upward bend in the mid-dorsal wall of the archenteron, and it
appears as if the notochord with the chorda-endoderm cells is
either pulling in or being pushed in from bordering the archen-
teric cavity, while the cells of the dorsal wall of the archenteron

on either side of the notochord are coming together under the notochord. A few sections beyond (Fig. 11), the notochord is almost entirely cut off from the archenteron, as only one or two heavily pigmented cells lie between the two parts of the dorsal endoderm. In the middle region of the embryo (Fig. 12), the endodermal cells have united under the notochord and the notochord is a rounded chord of cells entirely separated from the surrounding tissues.

In *Rana palustris*, therefore, as well as in *Bufo lentiginosus*, the notochord is composed entirely of mesoderm in the anterior part of the embryo, and of mesoderm and chorda-endoderm in the posterior region. The early stages in the formation of the notochord are very similar in the two species ; but in *Rana* there is no upward growth of yolk cells as in *Bufo* to form the permanent dorsal wall of the archenteron.

Most of the embryologists who have studied the early development of the Urodela agree with Jordan (10) who describes the formation of the notochord in the common newt as follows : " The cells of the median dorsal wall of the archenteron assume a somewhat columnar form and are gradually pushed up and pinched off until they are completely separated from the endoderm and come to lie above it in the mid-line." This view is held by Hertwig (7), Scott and Osborn (20), Field (5), Eycleshymer (4), Brachet (2), and Schwink (19).

Lwoff (13) is, perhaps, the most prominent of those who oppose this view. In his study of *Axolotl*, Lwoff finds that the mesoderm and the notochord are derived from cells invaginated from the surface of the egg at the blastopore rim, and he states : " Bei den Urodelen bildet sich die dorsale Wand des Darmes, ebenso wie bei Petromyzon, verhältnissmässig spät, nämlich nachdem die Chorda sich von den seitlichen Mesodermplatten gesondert hat. Die Entodermzellen wachsen von rechts und links einander entgegen, vereinigen sich unter der Chorda und bilden aufsolche Weise die dorsale Wand des Darmes." This description of the manner in which the permanent dorsal wall of the archenteron is formed in the *Axolotl* agrees remarkably well with the results of my investigations on *Bufo*. Lwoff's summary of the results of his study of the Anura based on an

investigation of the early development of *Rana*, is in part as follows : " Bei den Anuren liegen insofern anderen Verhältnisse vor, also hier die Zellen, welche die dorsale Wand des Darmes bilden, von Anfang an vorhanden sind ale eine Zellenreihe und zwar als eine untere Zellenreihe jener Anlage, aus welcher die Chorda entsteht." Lwoff and I are therefore in accord in believing that in *Rana* there is no upward growth of the yolk cells from the lateral walls of the archenteron to form the mid-dorsal wall.

There is great diversity of opinion concerning the manner of the formation of the notochord in the Anura ; and, considering the careful work that has been done in this line, it seems highly probable that the process is not as uniform in this group as it is in the Urodela.

Goette (6), from his study of the development of *Bombinator igneus* concludes that in this species a central chord of mesoblast in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord. This view is supported by the later investigations of Schultze (18), and Morgan (15) who worked on different species of *Rana*.

In a paper on the development of the middle germ layer in *Rana temporaria*, Hertwig (8) gives a number of figures of the posterior part of the embryo that bear a striking resemblance to those I have drawn of a similar region in the embryo of *Bufo lentiginosus*. Hertwig believes, however, that the entire notochord in the Anura as well as in the Urodela, is derived from a chorda-entoblast which at the sides of the archenteron pass into the endoderm cells forming the lateral walls. Field (5), from his investigations on *Rana temporaria* and on *Bufo vulgaris*, agrees with Hertwig regarding the manner of formation of the notochord, as do Robinson and Assheton (17) who worked on *Rana temporaria*. Balfour (1) also inclines to the same opinion, although he states that his evidence for so doing is not entirely conclusive.

As a result of his study of the early development of *Bombinator igneus*, Perenyi (16) advances still another theory regarding the formation of the notochord. He states that, when the blastopore closes in, " die vertikal nach innen vordringenden Zellen

der Deckzellen, welche zwischen beiden Teilen des Mesoderms liegen einander berühren und sich auf der dorsalen Seite von den äussersten Zellen abzuschnüren beginnen." In this way a rod of cells is cut off from the inner layer of ectoderm to become the notochord. I know of no other investigator whose results agree with those of Perenyi.

The results which Schwink (19) has obtained from his investigations on *Rana temporaria* and *Bufo vulgaris* are very similar indeed to those which I have recorded in the present paper for *Rana palustris* and *Bufo lentiginosus*. According to Schwink, the anterior portion of the notochord in *Rana temporaria* is entirely mesodermal in origin, while the posterior part has added to it a single layer of chorda-endoderm from the dorsal wall of the archenteron, the endoderm cells at the side of the notochord growing under and uniting in the mid-dorsal line. In *Bufo vulgaris* Schwink finds that the dorsal wall of the archenteron is composed of deeply pigmented cells which, at the sides of the archenteron, pass into the larger yolk cells, although he states that in some cases it appears " dass die hier liegenden Entoblastzellen aus dem bisherigen Verband scheiden um in den Mesoblast aufgenommen zu werden." Concerning the formation of the dorsal wall of the archenteron in the posterior part of the embryo Schwink states that, " hier von beiden Seiten Darmentoblastzellen gegen die Mittellinie streben und dass dadurch Zellen, die vorher den Darm dorsal auskleideten, mit zur Bildung der Chorda verbraucht werden." This agrees exactly with what I have found to occur in the posterior region of the embryo of *Bufo lentiginosus*.

Brauer's (3) studies on the development of the Gymnophiona show that, in the posterior region of the embryo, the upper wall of the archenteron is at first formed of cells which have been invaginated from the surface. These " animal cells " are sharply marked off from the yolk or " vegetative cells " which form the side walls of the archenteron. In the anterior part of the embryo, the archenteron is extended by its connection with the segmentation cavity which is bounded entirely by yolk cells. At an early stage of development, therefore, the dorsal wall of the archenteron in the anterior region of the embryo is composed of vegetative cells, while in the posterior region it is formed of cells invaginated

from the surface as I have found to be the case in the embryo of *Bufo lentiginosus.* At a later stage of development, vegetative cells grow up from the sides of the archenteron, and gradually cover up the invaginated animal cells which now form an unbroken sheet of mesoderm across the dorsal wall of the arch-enteron. A portion of this mesoderm in the mid-dorsal line is subsequently cut off from the lateral mesoderm to form the notochord.

In the posterior region of the embryo of *Bufo lentiginosus* a portion of the dorsal plate of cells which forms the mid-dorsal wall of the archenteron becomes cut off from the rest of the layer to be added to the notochord. If we attempt to trace the origin of this dorsal plate, we find that it is composed of cells invagi-nated from the surface of the egg before there was any division of the cells into ectoderm, mesoderm and endoderm. These invaginated cells form a part of the upper wall of the archenteron for a comparatively short period of development only, and those of the cells that are subsequently added to the splanchnic mesoderm soon lose their identity entirely, and cannot be distinguished in any way from the other cells of the mesoderm. The later his-tory of the chorda-endoderm cells I have not followed.

As the endoderm cells that grow up from the sides of the archenteron and meet under the notochord are unquestionably derived from the yolk portion of the egg, the archenteron even-tually becomes lined throughout its whole extent with yolk cells, and, therefore, the result is the same as if the archenteron was originally formed by a splitting between yolk cells as is believed to be the case by Robinson and Assheton (17), Houssay (9) and Moquin-Tandon (14).

According to Morgan, Wilson (21), Eycleshymer and others, there is an invagination of surface cells at the dorsal lip of the blastopore during the gastrulation of the frog's egg, and these invaginated cells come to form a part, if not all, of the dorsal wall of the archenteron in the posterior region of the embryo. In subsequent development, as the studies of Schwink and of myself show, these invaginated cells are not covered over by an upward growth of yolk cells from the lateral walls of the archen-teron as is the case in the toad embryo. A few of these cells

are added to the notochord, the rest, as far as I have been able to determine, remain as part of the permanent dorsal wall of the archenteron. I have never seen a section of an embryo that would warrant my stating that some of these cells become added to the mesoderm, although in the posterior region of the embryo endoderm and mesoderm are connected for a much longer time than they are in the embryo of *Bufo*.

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The Growth and Variability in the Body Weight of the Norway Rat (Mus norvegicus)

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

THE GROWTH AND VARIABILITY IN THE BODY WEIGHT OF THE NORWAY RAT (MUS NORVEGICUS)

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

THREE CHARTS

The investigations of Donaldson ('06, '09, '15), Slonaker ('12), Jackson ('13) and others have given a wealth of data regarding normal body growth in the domesticated albino rat. Information regarding the Norway rat, the wild prototype from which the Albinos were derived (Hatai, '07), is very meager, however, resting chiefly on the work of Miller ('11) and on scattered data noted by various investigators who were studying this species with the idea of exterminating it as a destructive pest ·and a menace to the health of mankind.

The present investigation was undertaken primarily for the purpose of obtaining norms for the Norway rat with which to compare the data for various series of hybrid and 'extracted' strains of rats. The growth and variability in the body weight of the first generation of Norways, born and reared in captivity, are described herein: subsequent papers will deal with litter production, the sex ratio, and the effects of domestication in this species.

MATERIAL AND METHOD

Wild Norway rats, trapped in various, localities near Philadelphia, were brought into the laboratory in February and in March, 1919. These animals, comprising sixteen males and twenty females, were all at least three months of age when captured. In spite of confinement and changed conditions of environment and of nutrition, they remained in good health for many months, though only six of the females were known to

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bear young.	After twenty-one litters, containing a total of 139 young, were obtained, the parents were killed and dissected by Doctor Donaldson, and they were found to agree, in all essential respects, with the many wild Norways which he and Doctor Hatai had examined at various times during the past ten years.

Norway rats breed at all seasons of the year, as Lantz ('10) and Miller ('11) have noted, but the period of most pronounced sexual activity seems to be in the early spring.	In captivity Norway females often devour their young at or soon after birth (Miller)—a tendency which these Norways exhibited in a marked degree.	After a number of litters had been destroyed, it became apparent that rats could not be obtained for this study if the Norway females were depended upon to rear their young.	The difficulty was overcome by removing the young Norways from the nest as soon as they were discovered and placing them with a lactating albino female.	Norway young evidently have a very different odor from that of albino young, since in some cases the albino female objected to rearing the Norways and either destroyed or neglected them.	If, however, the albino female was removed from the cage when the transfer of the young was made, and not replaced under half an hour, she usually accepted the foundlings and reared them as her own.	A total of 110 offspring of wild Norways were reared, and many of them were kept until they were two years old.	These animals, comprising 51 males and 59 females, form the material for the present study.

As it would seem to be an advantage in working with these Norways to have them tame, I made it a point to handle the young rats every day while they were with their foster mother. They submitted to this handling up to the time they were about thirty days of age, though they were very restless when held and usually tried to escape.	After weaning they began to show increased resentment at handling and attempted to bite.	The idea of taming them was abandoned, and subsequently they were slightly stupefied with ether when weighings were made.

The method used in obtaining the growth data for these Norways was the same as that employed in a former study of the growth and variability in the body weight of albino rats (King,

'15 a).   The individuals were weighed separately at birth, if they were obtained before they had suckled.   At thirteen and at thirty days of age the males and females of a litter were weighed collectively and the average weight for the two sexes recorded. After this time the rats were weighed separately, at intervals of thirty days, until they were two years old, when they were killed and the weight and condition of various organs determined.

The Norways lived under the same conditions of environment and of nutrition as the albino rats in our colony.   It was necessary, however, to protect the cages with wire netting at all vulnerable points, as the rats would gnaw their way through a thick plank in a few hours.   Although born in captivity, these Norways retained their inherent fear of man for many months. When the cage was approached the rats promptly went in hiding under the nesting material, and, if disturbed or the bedding removed, fear made them both reckless and savage, and they sprang at one's face if the cage door was opened.   If they escaped from the cage they fought viciously when captured, and when returned to the cage they ran wildly about for some time. After several months they became more reconciled to captivity, and for the most part remained quietly in the corner of the cage when they were fed or the cage was cleaned.

GROWTH IN BODY WEIGHT OF THE NORWAY RAT

According to Miller ('11), Norway young weigh, on the average, 6.4 grams at birth, the males being slightly heavier than the females.   In the four litters of this series that were obtained at the time of parturition the average weight of the twelve males was 5.34 grams; that of the fourteen females was 5.09 grams. The discrepancy between Miller's finding and mine may possibly be due to the fact that the Norways weighed by Miller had suckled before the weighings were made while those of my series were weighed immediately after birth.

Norway young seem to have a somewhat heavier birth weight than albino young, as a large number of determinations for Albinos show that the average birth weight of the males is 4.69 grams and that of the females is 4.5 grams (King, '15).   It is

possible that the heavier birth weight of the Norway rats is due
to the fact that in this form the gestation period is from 23½ to
25½ days (Miller), while in the albino strain the normal gestation
is about 22½ days.   Growth of the albino fetus is very rapid during
the later days of gestation, as Stotsenburg ('15) has shown, and
if the Norway fetus grows at the same rate as the albino fetus
the prolongation of the gestation period for even a single day
might be expected to increase materially the birth weight of the
young.

Although Norway females weigh less than the males at birth,
they grow more actively during early life, as at thirteen and at
thirty days of age their average body weight is greater than that
of the males (table 1).   After puberty (about ninety days) the
males, as a rule, are considerably heavier than the females at all
age periods for which records were taken.   Albino rats show a
weight relationship between the sexes much like that existing
in the Norway strain (Donaldson, '06; Jackson, '13; King, '15 a).

Chart 1 shows graphs for the weight increase with age of the
fifty-one males and fifty-nine females comprised in this series of
Norways.   The graph for the females runs slightly higher than
that for the males until the sixty-day period when the graphs
cross; beyond this point the graphs show increasing divergence
with advancing age.   Both graphs mount steadily upward until
the 700-day period, thus indicating that the Norway rat tends
to increase in body weight throughout adult life.   There is a
slight drop in the graphs at the end of the weighing period, but
this can doubtless be attributed to the fact that many of the
animals were suffering from so-called 'pneumonia,' as autopsies
showed.   During early life Norway rats are somewhat more
resistent to 'pneumonia' than are albino rats, but they seem to
lose this resistance after they are about eighteen months of age,
and under laboratory conditions few of them can be kept free
of this scourge in later life.

In order to compare the growth in body weight of Norway rats
with that of Albinos, charts 2 and 3 have been constructed from
the data given in table 1 of the present paper and from the data

in table 3 of a previous paper which show the growth of a series of stock albino rats comprising fifty males and sixty-seven females (King, '15 a). Both Norways and Albinos lived under similar housing conditions. The Albinos, however, were fed on a 'scrap' diet consisting of carefully sorted table refuse, while the Norways received the mixed ration (chiefly cooked cereals, meat, and fresh vegetables) which for sanitary reasons has replaced the former diet used in our colony. The two series contain approximately the same number of individuals and are probably fairly representative of the different stocks when reared under seemingly favorable conditions of environment and of nutrition.

Growth data collected by different investigators for various series of albino rats (Donaldson, '06; Slonaker, '12; Ferry, '13; Jackson, '13, et al.) all show that albino males grow very rapidly during the first 120 days of postnatal life, and that there is subsequently only a relatively slight increase in body weight. Males of the first generation of Norways born in captivity showed no marked acceleration in growth at any age period, as is indicated by the form of the graph in chart 2. Increase in body weight was steady and fairly uniform until the individuals were approaching senescence. In chart 2 the graph for the Norways crosses that for the Albinos at the 485-day period. No comparison can be made between the weight increase in the two forms at later age periods, as weight records for albino males have not been made, as yet, for any number of older animals.

Relative growth changes in body weight of Norway and albino females are similar to those for the males, as is shown by a comparison of the graphs in chart 3 with those in chart 2. In the case of the females, however, the graphs cross at an earlier age period (340 days), and subsequently the Norway females are heavier than the albino females at all age periods for which records are available (chart 3). Many of the Norway females are very heavy in later life, often weighing close to 400 grams— a weight that is rarely attained by any albino females.

The only observations, previously recorded, regarding the growth in body weight of Norways rats are those of Miller ('11) for a litter of eleven Norway young born in captivity. The

average body weight of these individuals, at thirteen days of age, sexes not separated, was 15.1 grams; at twenty-five days of age their average weight was 25.9 grams. These data are in accord with those given in the present paper.

TABLE 1

Data for eighteen litters of Norway rats, showing the increase in the weight of the body with age

AGE IN DAYS	MALES				FEMALES			
	Body weight in grams			No. indi-viduals	Body weight in grams			No. indi-viduals
	Average	Lowest	Highest		Average	Lowest	Highest	
13	15.4	12	21	51	16.3	12	21	59
30	33.4	26	41	51	37.0	27	45	59
60	81.9	49	144	51	75.2	47	110	59
90	115.9	68	201	51	100.5	70	152	59
120	148.6	70	245	51	122.6	77	184	59
151	176.1	87	284	51	142.0	98	201	56
182	195.9	110	300	51	153.5	111	256	54
212	218.4	128	330	51	172.1	117	272	50
243	235.4	140	374	50	189.3	133	278	52
273	253.5	154	379	50	204.2	131	327	51
304	265.5	156	380	50	209.8	123	301	47
334	276.7	170	395	50	221.5	128	345	49
365	287.3	184	428	48	232.8	152	344	47
395	297.4	185	442	48	242.2	135	383	47
425	312.2	200	457	47	247.5	156	380	43
455	322.3	188	471	47	260.5	172	359	44
486	332.0	226	459	46	257.9	182	382	43
516	335.0	226	476	43	264.1	163	373	37
547	345.6	231	486	41	261.5	173	375	36
578	358.0	233	516	39	266.5	168	360	34
608	361.8	231	518	38	271.9	184	339	31
639	369.9	236	540	37	279.7	190	367	30
670	374.5	240	546	37	284.4	210	355	26
700	364.2	243	518	34	283.4	200	376	25
730	362.1	231	505	27	278.9	197	341	19

It may be maintained that the slow growth of these Norways during the adolescent period is not normal for the species in its wild habitat, and that the form of the graphs shown in chart 1
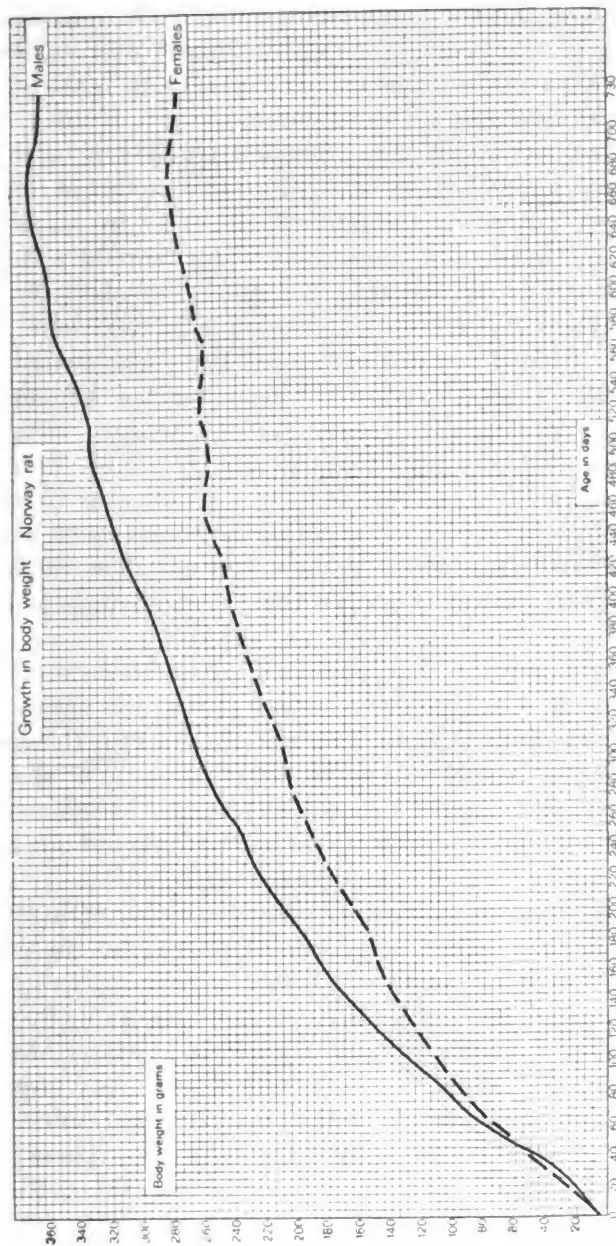
Chart 1   Graphs showing the growth in body weight of male and female Norway rats (data in table 1).

is the result of the conditions under which the animals lived. The rearing of the young Norway by albino foster mothers was not a factor that tended to retard early growth, as is shown by the following test that was made to determine this point. A litter of eight young born to a Norway female after she had been several months in the colony was separated into two groups of four each; one group was reared by the mother, the other by an albino female. At the thirteen-day period the young reared by the albino female weighed, on the average, 3 grams more than the young suckled by their mother. At thirty days of age there was an average difference of 4 grams in favor of the young reared by the albino female. During later life the individuals of the two groups showed no pronounced difference in body weight. Additional evidence that the suckling of the young by albino females did not retard the growth of these Norways is indicated by the fact that individuals in the second and third generations of Norways, that were all reared by their mothers, did not show the acceleration in growth during early life that is characteristic for the Albinos.

A second assumption that might be advanced to account for the slow growth of the young Norways is that the constant fear exhibited by the animals tended to inhibit their growth processes. Against such an assumption is the fact that some of the young Norways that were tamed by an assistant in the laboratory so that they showed no evidence of fear at handling did not grow at a more rapid rate than did the untamed animals.

It does not seem probable that the inherent growth tendency in the Norway rat would be greatly restricted by the environmental and nutritive conditions under which these rats lived, since these conditions would seem to be more favorable for growth than those under which the animals live in a wild state. The cages in which the Norways were confined were very large; food was varied and abundant, and the rats were not subjected to extremes of temperature nor harassed by enemies. These factors, it would seem, should serve to counterbalance any detrimental action of captivity on growth.
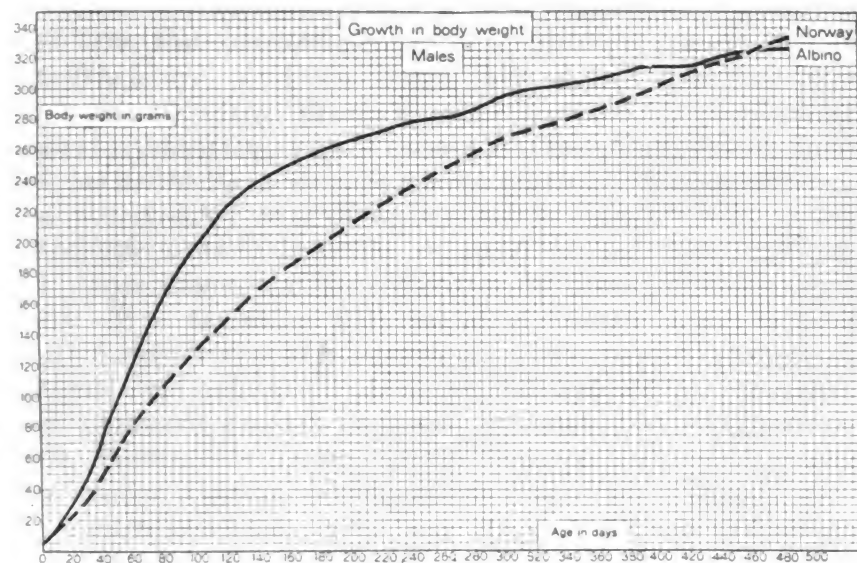
Chart 2   Graphs showing the growth in body weight of Norway and of stock albino males (data in table 1, and in table 3 of a previous paper; King, '15 a).
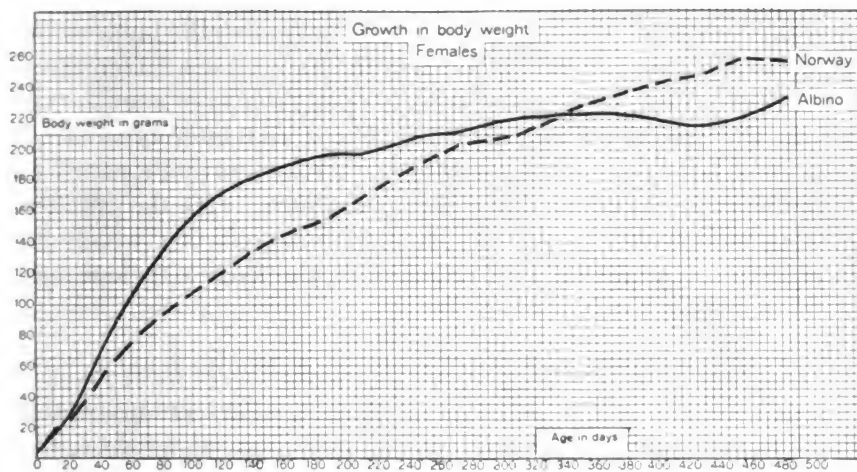
Chart 3   Graphs showing the growth in body weight of Norway and of stock albino females (data in table 1, and in table 3 of a previous paper; King, '15 a).

If the graphs in chart 2 and in chart 3 are fairly representative of the normal growth in body weight of Norway and of albino rats, then it is evident that growth energy is expended very gradually by the Norways and very rapidly by the Albinos. As rapid growth during early life seems to be characteristic of most domestic animals, it would appear that the acceleration in the growth of the Albinos is one of the effects of domestication on this form and that the Norways will tend to show the same acceleration in growth when they too become domesticated. In favor of this assumption is the fact that individuals in the later generations of these Norways grew somewhat more rapidly during early postnatal life than did those of the first generation. Graphs as plotted for animals in the second to the fifth generation are progressively more like those for the domesticated Albinos, and if the change in the form of the graphs continues, in a few more generations the Norways will show as great an acceleration of growth during adolescence as do the Albinos.

Not only in body growth, but also in other respects the Norways exhibit a slower development than do the Albinos. As Miller has stated, and as my observations show, Norway rats do not open their eyes before the sixteenth day of postnatal life (usually not until the beginning of the seventeenth day), while the eyes of albino rats are open when the animals are fifteen days old; in both forms the females tend to open their eyes a few hours before the males. Ping ('21) has found a relatively smaller number of large cells in the superior cervical sympathetic ganglion of the Norway rat at one day of age as compared with the number of such cells found in albino rats of like age—a fact which he interprets as indicating a retardation in the early development of these cells in the Norways.

VARIABILITY IN THE BODY WEIGHT OF NORWAY RATS

It is generally assumed that animals in a wild state are much more variable in many characters than are those under domestication, the variability being due, in great part, to environmental factors. Wild animals living in captivity under fairly uniform conditions of housing, of temperature and of nutrition might

therefore be assumed to lose much of the variability due to environmental causes and to exhibit chiefly that inherent in the species.

TABLE 2

Showing the coefficients of variation with their probable error for the body weights at different ages of a series of Norway rats (51 males and 59 females), and of a series of stock albino rats (50 males and 67 females)

AGE IN DAYS	NORWAY SERIES		ALBINO SERIES	
	Males	Females	Males	Females
13	12.2±0.82	14.6±0.90	11.8±0.79	11.4±0.76
30	13.0±0.86	17.3±1.07	10.2±0.68	11.0±0.74
60	24.1±1.60	19.8±1.22	17.0±1.14	15.7±1.05
90	26.2±1.74	16.9±1.04	14.8±0.99	12.5±0.95
120	28.2±1.88	16.0±0.99	13.4±0.90	10.3±0.75
151	24.2±1.61	16.6±1.03	13.3±0.89	10.4±0.73
182	23.4±1.55	17.8±1.13	14.2±1.22	12.3±0.90
212	21.8±1.45	17.9±1.20	14.0±0.96	12.4±0.91
243	23.7±1.60	18.2±1.26	13.9±0.99	12.6±0.91
273	22.4±1.50	19.1±1.27	13.4±0.99	11.5±0.89
304	21.5±1.45	19.9±1.38	14.0±1.11	10.3±0.79
334	19.8±1.33	20.6±1.40	13.7±1.13	10.8±0.87
365	19.5±1.33	20.4±1.41	13.0±1.16	10.7±0.91
395	19.5±1.34	22.3±1.54	12.6±1.22	11.5±0.98
425	17.6±1.22	21.1±1.47	13.4±1.32	10.9±0.94
455	19.2±1.32	18.9±1.35	13.6±1.67	8.9±0.99
486	17.3±1.21	19.5±1.41	15.0±2.06	13.4±1.77
516	17.4±1.26	18.3±1.43		
547	16.7±1.24	19.4±1.54		
578	18.3±1.39	16.4±1.34		
608	17.1±1.32	15.3±1.28		
639	17.4±1.36	15.8±1.37		
670	17.5±1.39	14.2±1.32		
700	17.7±1.36	11.9±1.14		
730	19.4±1.79	13.9±1.52		

The extent of variability in the body weights of these Norway rats was determined from a study of the coefficients of variation which were calculated for each age at which weighings were made. These coefficients, with their probable error, are shown in table 2, which also gives the coefficients of variation and probable error for the body weights of the series of stock Albinos with which the

growth of the Norways has been compared. The latter series of coefficients are reproduced from table 4 of a previous paper (King, '15 a).

Grouped data were used in calculating the coefficients of variation for the thirteen- and for the thirty-day periods in both Norways and Albinos, as only the average body weight for the rats of each sex in a given litter was recorded at these ages; individual data were used in obtaining the coefficients for all the other age periods noted.

In early postnatal life Norway females grow more rapidly than do the males and they are likewise more variable in body weight, judging from the relative size of the corresponding coefficients of variation (table 2). The variability in the body weight of the males has increased greatly by the time the animals are sixty days old, as is shown by the fact that the coefficient of variation for this period is nearly twice as large as that for the first period recorded. From this time until the rats are nearly a year old the males show a much more marked variability in body weight than do the females. Growth in body weight begins to slow down perceptibly in the females after 365 days of age (chart 1), yet from this time until the 547-day period the females are quite as variable in body weight as the males. The corresponding coefficients of variation for the two sexes covering this period show a difference in favor of the females, as a rule, yet these differences are, for the most part, within the limits of possible error and therefore are not significant. As old age approaches, variability in body weight decreases slightly in both sexes, but tends to be higher in the males than in the females.

In sixteen of the twenty-five age periods indicated in table 2 the coefficients of variation for the body weights of the males exceed those for the females; in eight of these cases the differences can be considered as significant, since they are more than three times the probable error. Where the coefficients for the females exceed those for the males the differences are too small to be of import. It would appear, therefore, that Norway males tend to be more variable in body weight than Norway females, especially during the period of early maturity. This finding agrees

with that for stock Albinos (Jackson, '13; King, '15 a), and accords with the view that males, in general, are more variable than females (Darwin, '71; Brooks, '83).

A comparison of the coefficients of variation for the Norways with corresponding coefficients for stock Albinos as given in the last two columns of table 2 shows that at all age periods Norway rats, both males and females, are much more variable in body weight than are albino rats.   The maximum variability for both sexes of Albinos comes at sixty days; in the Norways the maximum variability comes at a much later period, 120 days for the males and 395 days for the females.   In both forms the coefficients are relatively low for the first two age periods recorded; they rise to a maximum, and then decline somewhat as the animals approach senescence.

The results of this study support the view that variability is much greater in wild animals than in those under domestication. How much of this variability in the Norways is due to environmental factors and how much is inherent in the species remains to be determined.   Although albino rats have been living in a state of domestication for a long period, they show nevertheless an inherent tendency to variability in body weight at different age periods that is seemingly little affected by environmental conditions or by close inbreeding.   Series of albino rats reared under fairly uniform conditions of housing and of nutrition and inbred brother and sister for twenty-five consecutive generations showed, at the end of this time, a variability in body weight at different age periods that was but little less than the variability exhibited by outbred stock Albinos (King, '18, '19).   Whether the inherent tendency to variability in body weight is greater in the Norways than in the Albinos can be determined only after the Norways have become domesticated.

In man, according to Boas ('97) and Porter ('05), variability in body weight is correlated with rapidity of growth.   Such a correlation is indicated for the albino rat also according to the data given in the last two columns of table 2, since here the coefficients of variation for body weight are highest for the age periods at which growth is most rapid (sixty to ninety days), and

then drop sharply when the period of rapid growth is at an end. According to my records, Norway rats grow very slowly during early postnatal life, and it is doubtless significant that the coefficients of variation for body weights at this age are relatively low for both sexes. The growth in the body weight of Norway rats continues at a fairly uniform rate for a long period of time. During this period the coefficients of variation for the females rise steadily, and they begin to fall only when the females are nearing the end of the reproductive period and their body weight has reached the level which it retains as long as the animals are in good condition. In the Norway males the correlation between rate of growth and variability in body weight is not as marked as in the case of the female. The coefficients of variation are very large during early maturity when the animals are growing vigorously: they remain at a high level during later adult life while the animals are still steadily increasing in body weight, and show no marked drop up to the time the animals are two years of age. In both Norways and Albinos there seems to be a correlation between variability in body weight and age, variability being relatively low during early postnatal life, increasing rapidly at puberty, and continuing at a high level until the end of the reproductive period, when it drops near the level of early life. Variability in body weight is seemingly correlated both with rate of growth and with age in the rat and in man. In this respect, as in many others (Donaldson, '06, '18), life processes in these two widely separated forms are in close accord.

SUMMARY

This paper gives the results of a study of the growth and variability in the body weight of eighteen litters of Norway rats, comprising fifty-one males and fifty-nine females, that were the offspring of wild Norways trapped in the vicinity of Philadelphia. The data given cover the period from birth until the animals were two years old.

Norway rats breed at all seasons of the year, but the period of most pronounced sexual activity is in the early spring.

Norway males have an average birth weight of 5.34 grams; females are slightly smaller than the males, having an average weight of 5.09 grams at birth.

During early postnatal life Norway females have an average body weight greater than that of the males. After sixty days of age the males, as a rule, are heavier than the females at all age periods.

Norway rats do not show the marked acceleration in body growth during early life that is characteristic of the albino strain. Increase in body weight progresses at a fairly uniform rate in both sexes until the animals are approaching senescence, the increase in the adult state being greater in the males than in the females (chart 1).

Albino rats are much heavier than Norway rats during adolescence and early maturity; in later life the body weights of the Norways exceed those of the Albinos (charts 1 and 2).

It seems probable that the slow growth of the Norways during early life is characteristic for the strain, and that the acceleration in the growth of the Albinos is one of the results of domestication.

Norway rats show a high degree of variability in body weight at all age periods (table 2). In early life the females are somewhat more variable than the males, but in the adult state the males tend to be the more variable.

Norway rats at all age periods are much more variable in body weight than are albino rats (table 2).

In the rat, as in man, variability in body weight is apparently correlated both with rapidity of growth and with age.

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THE ANATOMICAL RECORD

CONTENTS

THE AMERICAN ANATOMICAL
MEMOIRS
No. 17

LIFE PROCESSES IN GRAY NORWAY RATS
DURING FOURTEEN YEARS IN CAPTIVITY

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

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THIRTEEN TEXT FIGURES AND TWO PLATES (THIRTEEN FIGURES)

CONTENTS

INTRODUCTION

Since 1919 a strain of wild gray rats (Rattus norvegicus) has been maintained at The Wistar Institute for the purpose of determining the changes that occur in these animals when bred in captivity for a number of generations. My colleague, the late Dr. Henry H. Donaldson, was cooperating in this investigation and tracing alterations in the body and organs, while I was studying changes in various life processes. A report on this work, dealing with data for the first ten generations born in captivity, was published some years ago (King and Donaldson, '29).

At the twenty-fifth generation the detailed study of rats in each succeeding generation was discontinued, and subsequently only individuals in every fifth generation were under observation. This generation, therefore, ends the period of captivity to be covered by a second report on this strain. Data for life processes in the eleventh to the twenty-fifth generation are given in this paper. Doctor Donaldson's records for organ changes in these generations will be published later.

Throughout the course of this investigation the housing conditions, care of animals, and methods of obtaining and recording data were not changed. Although food constituents varied somewhat at times, the rats always received a cooked ration, supplemented twice each week by raw vegetables and fruit. No attempts were ever made to stimulate growth and reproduction by the use of hormone extracts or special diets, nor were any individuals treated with x-rays, radium or other agencies in an endeavor to induce mutations. Thus the strain was maintained constantly under fairly uniform environmental and nutritive conditions that furnished adequate food, security from enemies, and protection from extreme temperatures.

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In this investigation, as in others continued over a long period, contingencies have arisen at times that had a marked effect on animals then under observation. Unfavorable conditions in the colony decreased fertility and the growth rate of individuals in the eleventh to the fifteenth generation. Similar effects followed removal of the colony to temporary quarters in 1932, and its subsequent transfer to a new location the following year.

Various subjects are discussed here in the same order as in the previous report, and a new section has been added dealing with the mutations that appeared in later generations. Many tables contain a summary of relevant data for the first ten generations in order to show successive steps in the series of changes that occurred in these rats during 14 years of captivity (1919–1933).

GROWTH IN BODY WEIGHT

Changes in the weight of the body with age were determined for 1674 individuals from the fifteen generations covered by this report, approximately the same number of rats being taken from each generation. To facilitate an analysis of the findings, data for five successive generations were combined into the three groups given in reference tables 1 to 3.

The body weight relations of the sexes during early postnatal life warrant consideration in view of the fact that in the first generation the average body weight of females exceeded that of males at 13 and at 30 days of age (King, '23), but in the second to the tenth generations weights of males exceeded those of females at all age periods (King and Donaldson, '29). A weight excess in young females, although similar to Donaldson's ('06) finding for young albino rats, does not accord with other determinations of the weight relations of the sexes in albinos (Jackson, '13; King, '15; Donaldson, '24).

When the first report on this strain was written sufficient data on the birth weights of gray rats had not been obtained to furnish the norms that are necessary in order to determine

the relative growth rate of the sexes during the suckling period. Such data are now available, since a recent paper (King, '35) gives birth weights for 6295 gray rats taken mainly from later generations. These records give the average weight of 3228 males as 5.5 gm., and that of 3067 females

TABLE 1

Increase in the weight of the body with age for individuals in the eleventh to the fifteenth generation of captive gray rats

| AGE IN DAYS | MALES | | | | FEMALES | | | |
| | Number of individuals | Body weight in grams | | | Number of individuals | Body weight in grams | | |
		Average	Highest	Lowest		Average	Highest	Lowest
13	269	18	25	14	278	17	23	13
30	269	44	78	28	278	42	75	26
60	269	89	149	49	278	80	142	47
90	269	136	247	83	276	114	184	74
120	269	168	290	96	269	144	208	90
151	267	199	308	127	260	168	253	105
182	265	225	343	141	259	185	271	115
212	263	244	358	152	258	197	277	129
243	262	263	395	162	265	208	306	145
273	257	276	433	171	258	219	340	152
304	254	291	452	209	263	229	344	164
334	254	302	441	186	265	238	355	172
365	253	310	471	220	250	246	330	178
395	250	322	464	236	251	254	343	183
425	249	333	490	245	251	260	387	190
455	242	336	479	250	245	268	391	189
486	239	348	490	252	231	277	415	186
516	234	357	502	269	226	288	433	182
547	224	365	497	282	228	292	417	197
578	214	375	547	265	217	298	398	220
608	209	379	553	289	203	302	404	219

as 5.2 gm. (King, '35). Thus at birth the body weight of gray males exceeds that of females by 0.3 gm. (5.5 per cent).

Taking these weights as norms, it is shown by data in table 1 that in the eleventh to the fifteenth generations the sexes were growing at approximately the same rate when 13 days of age. At 30 days, however, the weight difference of 2 gm. (4.5 per cent) indicates that females were then growing more

rapidly than males. In later generations (tables 2 and 3) the growth rate of females exceeded that of males throughout the suckling period, since at 13 days the average weights of males and females were the same, and at 30 days the weight excess of males was but 2 per cent. Subsequently the growth rate in females decreased while that in males increased. The

TABLE 2

Increase in the weight of the body with age for individuals in the sixteenth to the twentieth generation of captive gray rats

AGE IN DAYS	MALES				FEMALES			
	Number of individuals	Body weight in grams			Number of individuals	Body weight in grams		
		Average	Highest	Lowest		Average	Highest	Lowest
13	283	19	29	14	275	19	27	13
30	283	50	79	34	275	49	69	34
60	283	110	198	51	275	97	170	53
90	283	166	260	85	273	139	212	68
120	283	212	302	97	254	172	252	86
151	281	253	354	121	255	201	270	112
182	280	279	401	136	253	219	297	123
212	277	302	420	146	254	234	307	132
243	275	318	422	184	244	247	336	162
273	275	331	452	203	245	258	353	182
304	273	343	465	225	248	268	344	175
334	270	355	472	254	255	278	363	198
365	269	366	498	286	254	288	378	210
395	268	376	504	284	251	295	403	210
425	264	384	512	290	247	301	403	198
455	261	396	527	302	247	309	402	204
486	259	404	547	287	244	314	402	240
516	253	412	567	326	229	323	426	243
547	243	423	549	338	225	329	429	240
578	237	434	560	333	217	338	438	252
608	231	439	572	353	215	344	462	256

weight excess of males had increased to about 21 per cent by the time the rats were 8 months old. Thereafter the average weight differences between the sexes remained fairly constant. It thus appears that in gray rats, as in albinos (Donaldson, '06), the growth curves are similar to those for man in that, although males tend to be heavier than females at birth and

during adult life, females grow at a more rapid rate during early life. An acceleration in the growth rate of young females has been noted also in the guinea pig (Bessesen and Carlson, '23), in the mouse (Gates, '25) and in the cat (Latimer and Ibsen, '32), as well as in man (Baldwin, '14; Davenport, '26; Appleton, '28).

TABLE 3

Increase in the weight of the body with age for individuals in the twenty-first to the twenty-fifth generation of captive gray rats

AGE IN DAYS	MALES				FEMALES			
	Number of individuals	Body weight in grams			Number of individuals	Body weight in grams		
		Average	Highest	Lowest		Average	Highest	Lowest
13	280	18	28	14	289	18	25	15
30	280	48	89	35	289	47	78	34
60	280	109	180	53	289	91	160	58
90	280	174	276	94	284	147	216	83
120	280	216	309	137	265	183	253	110
151	279	249	354	179	269	208	286	129
182	279	275	385	188	273	225	305	137
212	279	297	400	203	276	239	335	144
243	279	314	442	239	273	252	345	164
273	278	327	445	251	271	264	352	175
304	276	341	446	264	276	276	368	186
334	274	352	445	262	260	282	387	203
365	272	369	458	304	266	289	368	203
395	272	372	482	298	257	295	387	212
425	267	382	515	305	260	299	382	230
455	263	396	518	312	256	306	388	245
486	257	400	524	303	252	310	405	251
516	249	412	554	347	252	321	416	246
547	247	423	550	353	247	329	418	277
578	234	424	551	356	240	338	440	283
608	225	441	553	364	227	342	447	285

Graphs in figure 1, constructed from data in tables 1 to 3, indicate the changes in body weight with age in successive generation groups of male rats and form a better basis for discussion than do the data in tables 1 to 3.

All graphs in figure 1 have approximately the same form, but graph 1 runs at a relatively low level due, in part, to the
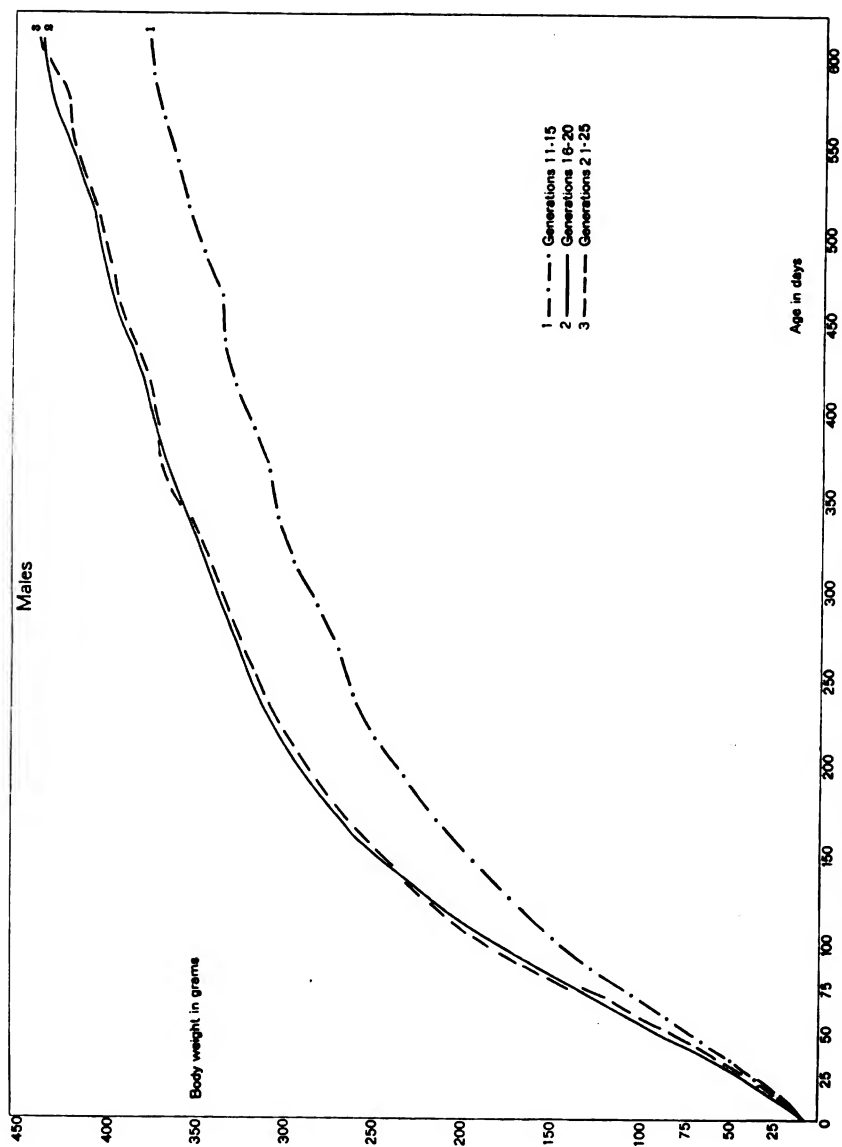
Fig. 1 Changes in the weight of the body with age in three generation groups of males.

fact that in individuals of this group growth was retarded by adverse conditions in the colony. That improved environmental and nutritive conditions had a favorable influence on body growth is shown by the rapid rise of graphs 2 and 3, which run very close together during their entire length. The difference in the level of the graphs during the latter part of their course indicates that adult males of the last two generation groups weighed about 15 per cent more than did those of the first group. The temporary check to body growth resulting from the transfer of the colony to a new location accounts for the depression near the end of graph 3.

Body weight changes with age in the three groups of females (tables 1 to 3) are shown graphically in figure 2.

While the graphs in figure 2 are similar in form to those in figure 1, they run at lower levels, as females tend to weigh less than males at all age periods after weaning.

Females of the last two generation groups responded to better environmental conditions by growing at a more rapid rate during early life, and attaining a larger size than did females of earlier generations. They weighed, on the average, about 13 per cent more during adult life than did females of the first group, as the levels of the graphs in figure 2 indicate.

According to Sorin ('32), litter size has a marked effect on the body growth of guinea pigs. This factor could not have influenced growth in gray rats, since throughout this investigation the great majority of litters used for study contained either five or six individuals: no litters of less than four or more than seven young were ever reared.

After twenty-five generations had been born in captivity, both rate and extent of body growth in males had increased greatly, as shown by the graphs in figure 3, which were constructed from body weight data given in table 5.

In figure 3 graph (A) rises gradually from the beginning to the end of its course, and shows no evidence of any pronounced growth acceleration during the adolescent period in males of the first generation. Body weight increase during later life was very uniform, and at the final weighing these
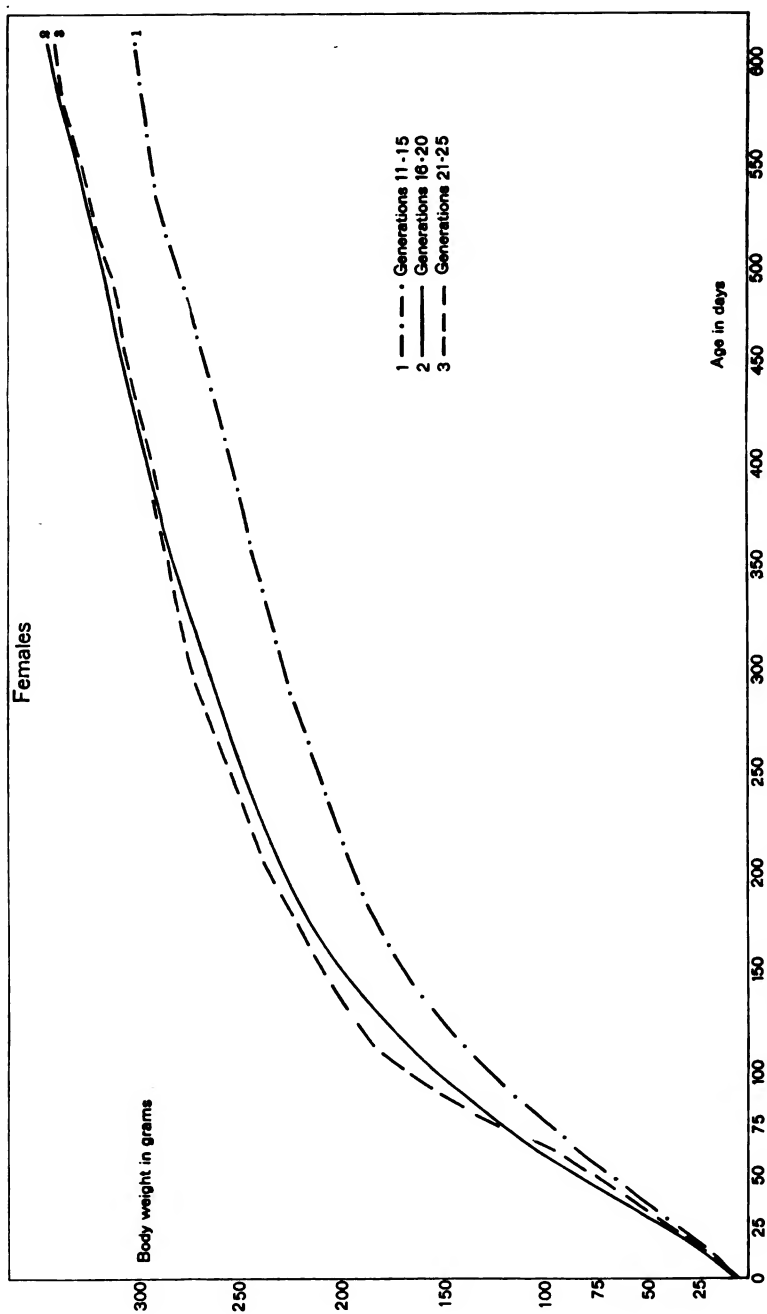
Fig. 2 Changes in the weight of the body with age in three generation groups of females.

males attained an average weight of 362 gm. Graph B, for
males of the twenty-fifth generation, rises rapidly and does
not tend to flatten until the age period of about 150 days, thus
indicating a marked growth acceleration during adolescence
and early maturity. The variance in the levels of graphs A
and B during the period of 60 to 150 days indicates a weight
difference between these two groups of males of some 35 per
cent. At subsequent age periods the graphs run nearly
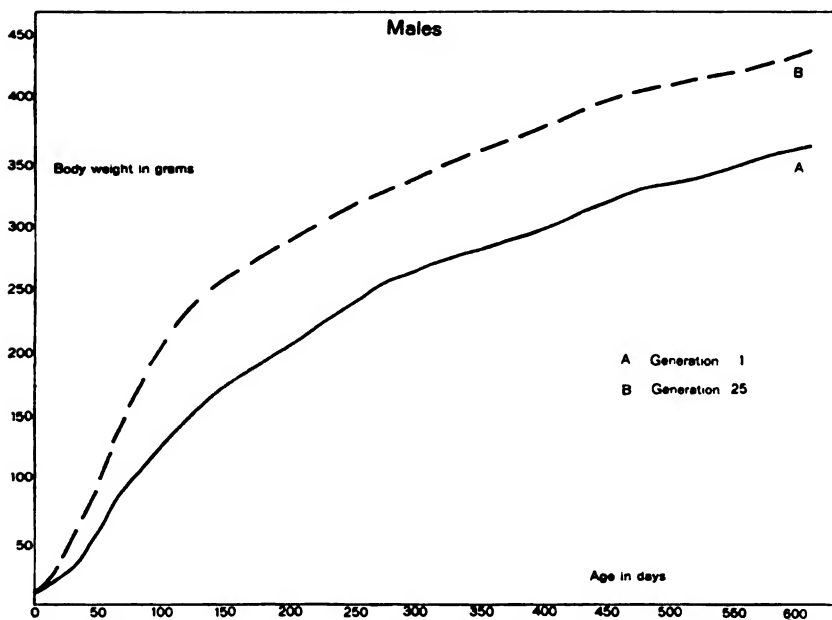
Fig. 3   Body growth in males of the first and of the twenty-fifth generation.

parallel, but at levels indicating that males of the last genera-
tion were about 20 per cent heavier than those of the first
generation during the greater part of adult life, and 17 per
cent heavier at the final weighing when their average weight
was 440 gm.

Data from table 6 were used in constructing the growth
graphs for females of the first and of the twenty-fifth genera-
tion, which are shown in figure 4.

The graphs in figure 4 run more irregularly than do those
in figure 3, because the effects of early pregnancy and of

lactation on body weights could not be eliminated nor offset
in compiling the data. The course of these graphs indicates
that females of the twenty-fifth generation grew much more
rapidly during early life, and were heavier at all subsequent
age periods, then females of the first generation, their weight
excess at the end of the weighing period being about 19 per
cent.

At the twenty-fifth generation, both sexes of gray rats were
growing during adolescence and early maturity at a rate
approaching that characteristic of albino rats maintained in
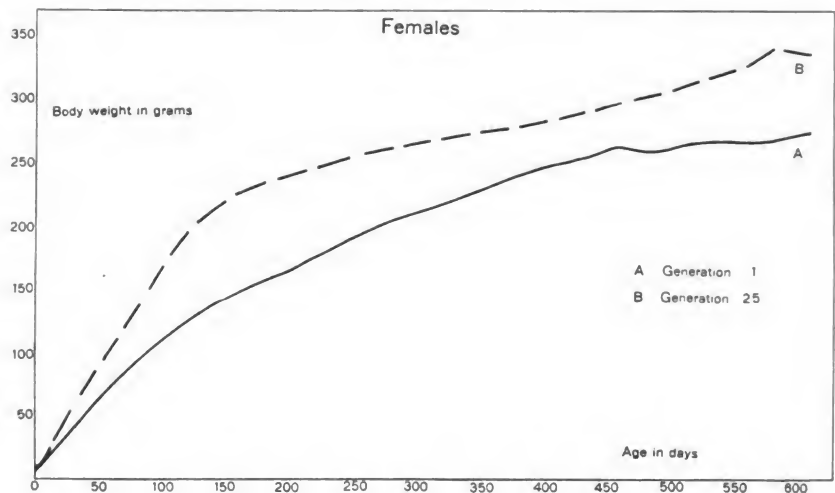
Fig. 4   Body weight in females of the first and of the twenty-fifth generation.

captivity for a long period of time. The average body weights
of adult grays, however, were greater than those of albinos
of like ages. The effects of captivity on the rate and extent
of body growth in gray Norway rats are similar to those that
have occurred in various domesticated mammals, such as
cattle, horses, and sheep. These animals, as Darwin (1875)
has shown, grow more rapidly and attain a much larger size
than did their wild prototypes. This growth increase in
domesticated animals is due, in all probability, to selective
breeding and to the more favorable conditions of environment
and of nutrition that captivity entails.

Important experimental work during recent years has shown that body growth in rats can be accelerated, and also greatly increased, by feeding nutritional diets or by the use of growth promoting hormones (Robertson, '16 a; Evans, '24; Osborne and Mendel, '26; Mendel and Cannon, '27; Smith and Bing, '28; Anderson and Smith, '32; Mendel and Hubbell, '35, etc.). To what extent other life processes are affected by these agencies has received little attention, as yet. Whether rapid growth during early life is beneficial to the individual and tends to a more active reproductive life and to longevity has long been questioned. Precocious body growth in man has never been deemed advantageous, since it seems to render individuals prone to certain diseases and to shorten the life span. It has been suggested that the difference in the growth rate of the sexes may be responsible for the fact that women tend to live longer than men, even when the latter have not been engaged in hazardous occupations that are liable to shorten life.

The relation between growth rate and longevity in albino rats has been investigated by McCay and his associates (McCay, '33; McCay and Crowell, '34; McCay, Crowell and Maynard, '35). These investigators found that individuals in which growth was retarded for a number of months by restricting the diet still retained the power to grow when they were old if then they were given adequate food. They did not, however, attain the size of animals that had grown rapidly during early life. Individuals with a retarded growth rate lived longer than did the controls, and females had a longer life span than males. McCay ('33) states: "No one has ever found it possible, however, to have both rapid growth with early attainment of maturity and longevity: It is possible that longevity and rapid growth are incompatible and that the best chance for an abnormally long life span belongs to the animal that has grown slowly and attained a late maturity."

The greater growth acceleration during early life and larger adult size of gray rats in later generations had no apparent detrimental effects on other life processes. On the contrary,

as shown by data in various tables, these rats attained maturity earlier than did those of preceding generations, their fertility was greater, the reproductive period longer, mortality during early life much less, and the average life span prolonged. There is no evidence, however, to warrant an assumption that rate and extent of body growth in these rats were precocious. Possibly growth was approaching the optimum for the race when individuals are maintained under environmental and nutritive conditions favorable for the full expression of growth activators without overstimulating them.

Although intrauterine factors doubtless play a major role in determining the growth of fetal young, rate and extent of postnatal growth, if environmental and nutritive conditions are uniform, must depend mainly upon growth potentialities which have their basis in the genetic constitution of the individual. It has been shown for the guinea pig (Bessesen and Carlson, '23), the rabbit (Kopéc, '26), the mouse (Kopéc, '29) and the rat (Dunn, '08; King, '16) that the early postnatal growth of individuals of the same litter is usually in the order of their birth weights. Individuals that are large at birth tend to grow more rapidly and to attain a larger size when adult than do those with a low birth weight.

Rats much below normal weight at birth, so-called runts, never attain the body size of other members of the litter even when reared under nutritive conditions that normally induce rapid growth (King, '16). Runts, apparently, are individuals in which growth processes have been retarded by some inherent factor. Their inability to grow at a normal rate cannot be due merely to nutritional handicaps during fetal life, since restriction of growth by malnutrition does not deprive normal rats of the power to grow when the diet is again adequate, as McCay, Crowell and Maynard ('35) have shown.

Another type of individual in which body size during adult life is as much above the norm as that of the runt is below it has been found in this strain of captive gray rats. Such rats are not noticeably large at birth, and they grow during early life at about the same rate as other individuals of the same sex in the litter. To show the course of body growth in

these individuals, growth data were compiled for twenty selected pairs of rats of each sex belonging in the sixteenth to the twenty-fifth generations. Each pair of rats of the same sex, one large and one small, were members of the same litter. All were individuals that continued to increase in weight throughout adult life, and were apparently in good physical

TABLE 4

Increase in body weight with age for twenty pairs of males and of females from the sixteenth to the twenty-fifth generation of captive gray rats. Each pair of the same sex (one large and one small) from the same litter

AGE IN DAYS	MALES		FEMALES	
	Average body weight in grams		Average body weight in grams	
	Large	Small	Large	Small
30	52.2	51.1	47.6	47.2
60	113.7	106.7	104.4	103.6
90	187.9	176.1	155.4	147.8
120	229.9	214.9	187.5	183.1
151	264.4	254.8	209.5	208.8
182	286.9	272.8	229.6	224.9
212	305.1	294.5	247.0	239.9
243	314.7	306.3	258.8	252.8
273	330.8	318.5	277.2	261.0
304	342.2	327.3	287.2	269.0
334	363.7	340.9	307.3	281.4
365	378.8	354.2	308.6	287.8
395	404.0	366.1	330.0	294.9
425	427.9	377.6	333.3	298.8
456	444.8	390.7	337.6	311.3
486	465.3	401.2	357.1	312.3
516	488.2	412.8	365.5	321.2
547	502.2	427.1	380.3	325.5
578	517.8	431.3	400.0	328.8
608	528.1	431.8	418.1	332.9

condition at the end of the weighing period. Differences in their body weights, therefore, cannot be attributed to disease. The smaller individuals of the pairs were not runts, nor was their growth restricted, as is shown by the fact that their average body weights at any age period were not significantly lower than corresponding weights for all rats in the generation groups from which they came (tables 2 and 3). Body weight increase with age in these rats is given in table 4.

The data in table 4 are shown graphically in figure 5.

In figure 5 the graphs for males (1 and 2) run parallel and at nearly the same level until the age period of about 300 days. Up to this point the space between the graphs represents a weight difference in the two groups of approximately 4.5 per cent, the weights of the smaller individuals being taken as the standards in computation. Subsequently the two graphs
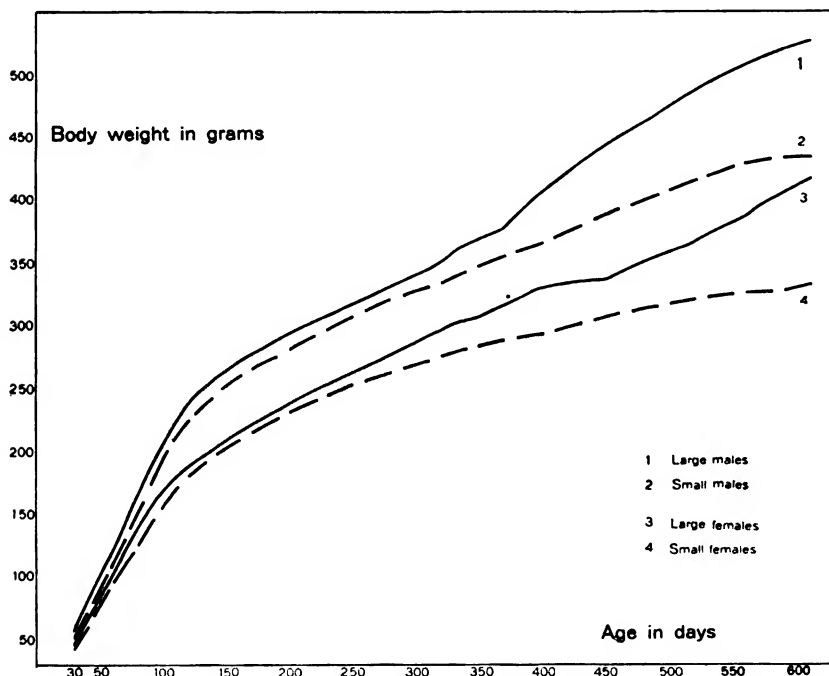
Fig. 5 Body growth in pairs of large and small individuals from the same litter.

diverge sharply; graph 1 mounts upward rapidly, graph 2 rises at a slower rate. At the 400-day period the larger males had increased their weight excess to 10 per cent, and they were 22.3 per cent heavier at the end of the weighing period.

Growth graphs for the two groups of females (3 and 4) run much the same as those for males, although they begin to diverge at an earlier age period. At 212 days the space between the graphs represents a weight difference between the

two groups of about 3 per cent. At 300 days this difference has more than doubled. The weight excess of the larger females was 11.9 per cent at 400 days and had become 25.6 per cent at the last weighing.

Autopsies made on a number of these large and small individuals disclosed no conditions that would account for the difference in their size. In the large individuals the body had increased as a whole and there was no evidence of differential growth in any of the organs.

As the number of these exceptional individuals increased as the generations advanced, it seems probable that the condition is inherited, and that these rats contained some genetic factor, or factors, that acted mainly during adult life to supplement the action of growth activating agencies. Genetic factors are known to play an important role in inciting and regulating growth processes, and their action is probably physiological, since there is no evidence, as yet, of the existence of specific size genes in mammals. Green ('31, '33) found that genes producing brown and dilute color in mice also increase the body size of the individuals. In confirming Green's finding, Castle, Gates and Reed ('36) have given evidence indicating that these genes influence body size "by virtue of their own physiological action."

Some of these large individuals became exceedingly savage and often severely injured or killed other inmates of the cage. Males with this 'killer' trait did not molest females of any age, but attacked adult males smaller than themselves and young males approaching maturity. Vicious females usually harmed only weaker individuals of their own sex. The 'killer' trait in these rats was doubtless a heritage from their wild ancestry, associated with the polygamous condition common to many animals living in their natural habitat.

Individuals much larger than their litter mates of the same sex have appeared occasionally in all strains of rats that have been under investigation in the colony during the past 20 years. 'Killers' have been found, however, only in captive grays and in mutant strains, particularly in mutant albinos.

VARIABILITY IN BODY WEIGHT

Body-weight variability in both sexes of gray rats changed considerably as the generations advanced. To indicate the trend and extent of this change, coefficients of variation for body weights at different age periods in individuals of three generations are given in tables 5 and 6. In these tables the series of coefficients for the first generation are reproduced from the former report on this strain (King and Donaldson, '29). Coefficients for the age period of 13 days were calculated from the average weights of males and females in each litter, as were also those for the 30-day period in the first generation. For all other age periods, the coefficients were calculated from individual data.

Coefficient of variation for body weights of males are given in table 5, those for females in table 6.

Tables 5 and 6 are given for reference only, since a graphic representation of the data for the first and for the twenty-fifth generations (fig. 6) shows more clearly the marked changes that occurred in the body-weight variability of gray rats during the period of captivity covered by the present report.

In figure 6 graphs (A and C) for body-weight variability in the two generation groups of males have the same general trend in that each rises to a maximum at an early age period, and then declines. Graph C, however, is much below graph A at all points, and its level as age advanced indicates that body-weight variability in males of the twenty-fifth generation was less than half of that in males of the first generation during the major portion of adult life, and even lower near the end of the weighing period when senility was beginning.

Graphs B and D, depicting body-weight variability in females of the two generation groups, run more irregularly than do those for males, because maximum variability in females of the first generation did not come until the rats were 13 months of age. For age periods up to 4 months differences between the graphs are not statistically important, but subsequently the levels of these graphs indicate that body-weight

TABLE 5

Coefficients of variation for body weights in three generations of captive gray males

AGE IN DAYS	FIRST GENERATION			TWENTIETH GENERATION			TWENTY-FIFTH GENERATION		
	Number of individuals	Average body weight in grams	Coefficients of variation	Number of individuals	Average body weight in grams	Coefficients of variation	Number of individuals	Average body weight in grams	Coefficients of variation
13	51	15	12.2±0.82	56	19	15.9±1.01	50	18	6.3±0.43
30	51	33	13.0±0.86	56	50	12.3±0.78	50	56	17.1±1.15
60	51	82	24.1±1.60	56	114	15.5±0.99	50	124	17.7±1.13
90	51	116	26.2±1.74	56	168	17.4±1.11	50	188	20.3±1.37
120	51	149	28.2±1.88	56	223	16.9±1.08	50	231	14.8±0.99
151	51	176	24.2±1.61	56	260	16.8±1.08	50	256	13.0±0.88
182	51	196	23.4±1.55	55	288	15.9±1.02	50	274	11.6±0.78
212	51	218	21.8±1.45	55	309	14.5±0.93	50	293	9.9±0.66
243	50	235	23.7±1.60	55	330	13.3±0.85	50	312	8.8±0.60
273	50	254	22.4±1.50	55	342	12.8±0.82	50	325	8.3±0.56
304	50	266	21.5±1.45	55	362	11.9±0.77	50	340	8.1±0.55
334	50	277	19.8±1.33	55	372	10.8±0.69	50	353	8.0±0.54
365	48	287	19.5±1.33	55	386	10.1±0.65	50	364	7.3±0.49
395	48	297	19.5±1.34	55	399	9.6±0.62	50	377	6.3±0.43
425	47	312	17.6±1.22	52	404	9.7±0.64	50	390	5.4±0.37
455	47	322	19.2±1.32	51	407	8.5±0.56	49	401	5.1±0.35
486	46	332	17.3±1.21	51	412	8.8±0.59	49	407	4.5±0.30
516	43	335	17.4±1.26	49	417	9.9±0.78	47	413	4.1±0.28
547	41	346	16.7±1.24	47	425	9.3±0.65	46	420	3.7±0.26
578	39	358	18.3±1.39	46	436	9.2±0.64	45	428	3.6±0.25
608	38	362	17.1±1.32	46	432	9.1±0.64	43	438	5.7±0.42

TABLE 6

Coefficients of variation for body weights in three generations of captive gray females

AGE IN DAYS	FIRST GENERATION			TWENTIETH GENERATION			TWENTY-FIFTH GENERATION		
	Number of individuals	Average body weight in grams	Coefficients of variation	Number of individuals	Average body weight in grams	Coefficients of variation	Number of individuals	Average body weight in grams	Coefficients of variation
13	59	16	14.6±0.90	53	19	15.8±1.03	51	18	5.5±0.37
30	59	37	17.3±1.07	53	49	14.7±0.96	51	52	16.3±1.09
60	59	75	19.8±1.22	53	97	16.9±1.10	51	109	17.5±1.17
90	59	101	16.9±1.04	52	138	17.9±1.18	50	158	12.9±0.87
120	59	123	16.0±0.99	48	179	13.8±0.95	47	195	13.5±0.88
151	56	142	16.6±1.03	50	206	12.2±0.82	50	218	11.9±0.80
182	54	154	17.8±1.13	48	225	11.8±0.81	47	232	11.8±0.82
212	50	172	17.9±1.20	49	243	10.6±0.72	49	241	10.9±0.68
243	52	189	18.2±1.26	45	257	11.7±0.83	49	250	10.2±0.86
273	51	204	19.1±1.27	49	265	10.1±0.81	46	261	9.8±0.69
304	47	210	19.9±1.38	46	278	10.0±0.70	49	265	8.4±0.57
334	49	222	20.6±1.40	49	288	8.5±0.58	47	270	8.1±0.56
365	47	233	20.4±1.41	50	301	9.1±0.61	49	277	7.5±0.51
395	47	242	22.3±1.54	46	307	9.3±0.65	46	283	6.7±0.47
425	43	248	21.1±1.47	48	315	9.1±0.63	48	289	6.9±0.48
455	44	261	18.9±1.35	47	319	8.7±0.60	47	297	6.5±0.46
486	43	258	19.5±1.41	46	324	8.7±0.61	48	302	6.1±0.42
516	37	264	18.3±1.43	44	328	9.1±0.65	42	317	6.7±0.49
547	36	262	19.4±1.54	44	337	9.9±0.71	42	322	6.4±0.47
578	34	267	16.4±1.34	42	343	9.2±0.68	44	340	7.5±0.54
608	31	272	15.3±1.28	39	344	11.3±0.86	43	337	7.7±0.56

variability in females of the twenty-fifth generation was sig-
nificantly less than that in females of the first generation.

A correlation between body-weight variability and the
growth rate in both sexes of gray rats is indicated by the fact
that in all generation groups of males (table 5) and in the
last two generation groups of females (table 6) variability
was at its maximum when the rats were young and growing
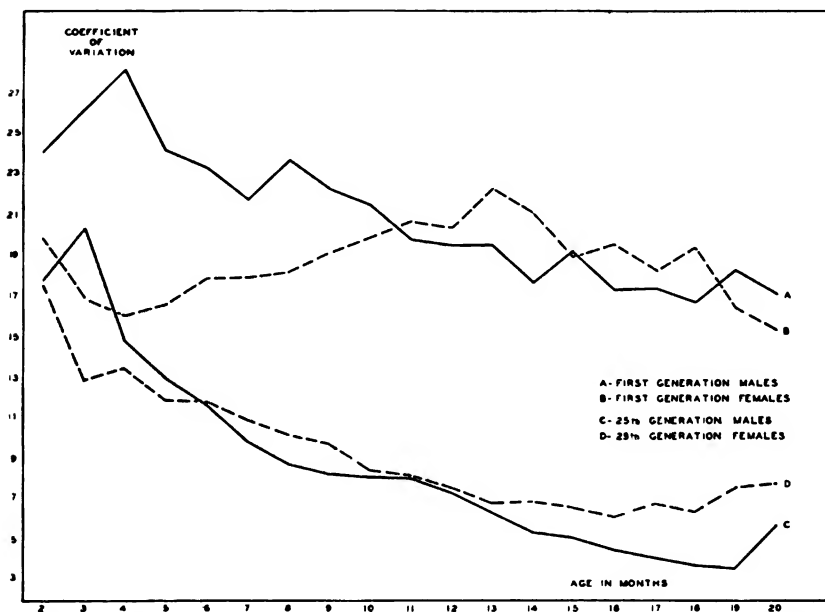
Fig. 6 Body-weight variability with age in the first and in the twenty-fifth
generation.

rapidly, and then decreased as the growth rate declined dur-
ing adult life. A similar correlation has been found in the
albino rat (King, '15), in the mouse (Robertson, '16; Saller,
'27; Kopéc, '32), as well as in man (Boas and Wissler, '05).

There is no sex difference in body-weight variability at
birth in gray rats. Coefficients of variation, calculated from
a large series of birth weights (King, '35) give for males a
value of 13.7 ± 0.11, and for females 13.6 ± 0.12. Compari-
son of corresponding coefficients in tables 5 and 6 and the

graphs in figure 6 show, however, that males tended to be more variable than females during early postnatal life, and less variable during later life. It seems probable that the greater body-weight variability of gray females during adult life can be ascribed to reproductive activity, as Kopéc ('32) has suggested is the case in mice. Pregnancy and lactation have marked effects on the body weights of breeding females. When weight determinations are made at stated intervals over a considerable period of time, the data obtained often include weights for lactating females and for those in early stages of pregnancy, even though, as in this investigation, weighings are omitted when females are known to be pregnant or when their weights are greatly reduced because they are suckling large litters. Coefficients of variation for body weights of adult females, calculated from data thus obtained, are undoubtedly larger than would be a series of coefficients based on data for unmated females whose weights had not been affected by reproductive processes. Taking this fact into consideration, the greater body-weight variability of females during adult life has no significance. For age periods during early life, where the two series of data are strictly comparable, body-weight variability in males was somewhat greater than that in females. A similar sex relation in body-weight variability has been found in albino rats (Jackson, '13; King, '18, '19) and in mice (Robertson, '16; Saller, '27; Kopéc, '32). These findings accord with the view that throughout the organic world males, in general, tend to be more variable than females.

Darwin (1875) was of the opinion that variability of every kind is caused directly or indirectly by changed conditions of life, and that, "if it were possible to expose all individuals of a species during many generations to absolutely uniform conditions of life there would be no variability." In light of our present knowledge of genetics, it is probable that changed conditions of life in captivity had little effect on the body-weight variability of gray rats. The gradual decrease in the variability of these rats as the generations advance can

doubtless be ascribed mainly to the method used in selecting individuals to propagate the race and to inbreeding.

All individuals in this strain of rats descended from six pairs of wild rats. No new stock has ever been added. Except in the descendants of one pair of feral animals, few matings were made in early generations between individuals that were very closely related. Rigid selection of only the most vigorous animals as parents of the succeeding generations gradually eliminated descendants of some of the wild rats, thus rendering the stock more homogeneous. For reasons given in the final section, all individuals in the twenty-first to the twenty-fifth generations were inbred, brother and sister. This close inbreeding undoubtedly tended to reduce variability and so is responsible, in great measure, for the low body-weight variability in rats of the twenty-fifth generation.

THE REPRODUCTIVE PERIOD

When wild animals are brought into captivity, the most marked effect of the changed conditions of life, at first, is on their reproductive activity. Some wild forms never breed after being removed from their natural habitat; others produce but a small number of young which often must be reared by foster mothers since their own mothers do not care for them. In most cases a considerable period of time elapses before individuals become sufficiently adjusted to their new environment to produce and rear strong, vigorous offspring. The cause of this low fertility in wild animals when first captured is unknown, and, as Darwin (1875) has stated, "we can only infer that it is caused by a change of some kind in the natural conditions of life."

Thirty-six wild rats (sixteen males and twenty females) were brought to the laboratory to serve as foundation stock for this strain of captive grays. Judging from their size, the youngest of the females were at least 3 months of age when captured, the oldest being probably a year old. None of the females were pregnant when captured, although all of them

were vigorous and seemingly free from disease. The changed conditions of life to which these rats were subjected affected reproductive processes adversely, causing sterility in some females and greatly reducing fertility in others. Only six of the wild females bred in captivity, and the litters they cast were small. Under their new environment wild females, with one exception, seemed incapable of suckling their offspring, and their litters were either destroyed soon after birth or neglected. In order to save the young it was necessary to remove them from the nest soon after their birth and give them to lactating albino females to rear. Restrictions to breeding resulting from change of habitat began to disappear in individuals of the second generation, and subsequently the great majority of females reared were fertile and able to rear their young.

It has been stated (Eaton and Stirrett, '28) that wild rats become sexually mature when they are about 3 months of age, but this statement lacks confirmation. In light of the breeding data obtained from trapped wild rats and from individuals in the early generations of captive grays, I am inclined to the opinion that gray females living under natural conditions do not begin to breed, as a rule, until they are at least 4 months of age.

With the advance of the generations there was a marked increase in the length of the reproductive period, as is shown by the data in table 7.

In later generations, as in the earlier ones, there was considerable variation in the time at which individual females began breeding. While some females cast their first litters when less than 3 months of age, the majority of them did not breed until they were at least 4 months old. The average age of females at the onset of breeding decreased steadily as the generations advanced, and was but 119 days at the twenty-fifth generation (table 7). Thus, at the end of the period of captivity covered by this report, the reproductive life of females began 147 days earlier than did that of females in the first generation whose average age at the inception of

breeding was 266 days. Generation changes in the age of females at the beginning and at the end of reproductive life are indicated by the graphs in figure 7.

The earlier breeding of females in late generations can be ascribed, in part at least, to their more rapid growth during adolescence. As a rule, rats that grow rapidly when young are the ones that begin to breed at an early age; those that

TABLE 7

Length of the reproductive period in the tenth to the twenty-fifth generation of captive gray rats

GENERA-TION OF BREEDING FEMALES	EARLIEST AGE AT WHICH A LITTER WAS CAST	LATEST AGE AT WHICH A LITTER WAS CAST	TOTAL LENGTH OF REPRO-DUCTIVE PERIOD	AVERAGE AGE WHEN FIRST LITTER WAS CAST	AVERAGE AGE WHEN LAST LITTER WAS CAST	AVERAGE LENGTH OF REPRO-DUCTIVE PERIOD
	days	*days*	*days*	*days*	*days*	*days*
10	101	692	591	207	490	283
11	93	642	549	210	509	299
12	90	730	640	175	506	331
13	108	652	544	169	510	341
14	130	784	654	209	548	339
15	99	802	703	172	521	349
16	104	728	624	195	559	364
17	78	646	568	137	522	385
18	87	665	578	151	546	395
19	75	657	582	135	547	412
20	88	655	567	136	512	376
21	95	731	636	143	535	392
22	79	655	576	124	525	401
23	84	617	533	128	522	394
24	88	643	555	140	540	400
25	76	667	591	119	559	440
10–25			593	159	528	369
1–9			517	226	453	227

grow slowly do not mature until much later. It is possible, also, that in the rat, as in the domestic fowl (Warren, '34; Hays, '36) age at sexual maturity is an inherited character that can be influenced by selection. Assuming that some individuals in the foundation stock of this strain carried genes tending to induce early maturity, such genes would have accumulated in the stock after a time because of inbreeding. The

majority of litters reared in later generations were cast by
females that had grown rapidly and bred at an early age, but
selection of litters to continue the strain was not based on the
early breeding of the mothers, but on the size and vigor of
the young at birth, the number of individuals in a litter, and
its sex composition.

In rats maintained under laboratory conditions reproduc-
tion ends, usually, when the animals are about 18 months of
age, although cases are known where young were born when
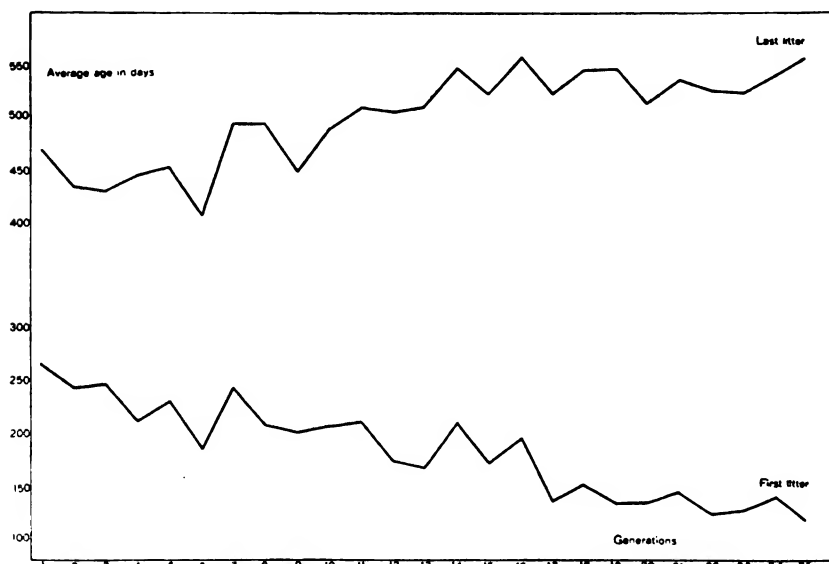
Fig. 7   Average age of females when the first and the last litters were cast.

females were over 2 years old.   The time at which the meno-
pause appears depends largely upon the physical status of
the females, especially on their ability to resist pneumonia.
This infection soon ends reproductive activity, although af-
fected individuals may live for some months.

The age at which individual females in later generations
stopped breeding varied greatly, the range being 185 days
(table 7).   The average age of females at menopause tended
to advance with the generations, as is indicated by the course
of the upper graph in figure 7.

The better physical condition of the rats after they had been in captivity for several years, and the less frequent occurrence of tumors and infections of the genital tract, probably account in great measure for the extension of reproductive life. There is also a possibility that heredity may be a factor involved in defining the limits of reproductive activity, since in some cases relatively young females, seemingly in good physical condition, cast only two or three litters; in other cases all females of a given litter produced approximately the same number of litters and stopped breeding at about the same age.

Changes in the average length of the reproductive period in females of the first twenty-five generations are shown by the graph in figure 8.
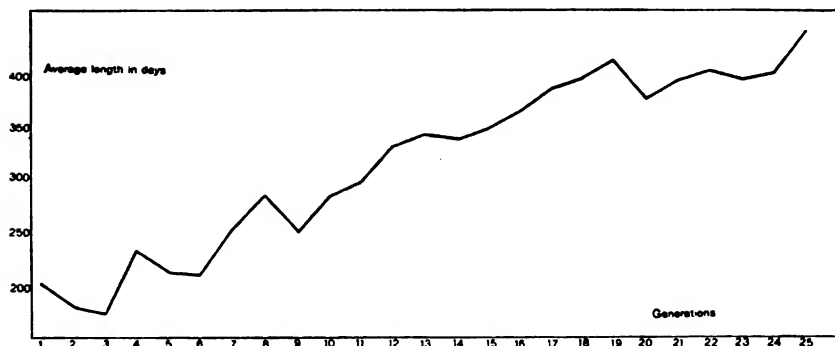
Fig. 8 Average length of the reproductive period in different generations.

Although there are irregularities at the beginning of the graph in figure 8, its general trend is upward. There are noticeable depressions at the ninth and twentieth generations, ascribable to the fact that in these generations breeding was checked because a number of females had ovarian cysts or developed pneumonia at an early age. The difference in the level of the graph at its beginning and the level at its end indicates that, at the twenty-fifth generation, the average length of the reproductive period (440 days) was more than twice that (204 days) in the first generation. This extension of reproductive life and also the increased fertility of captive gray rats, which will be shown in the following section, accord

with the findings of Darwin (1875) and others that under the more favorable environmental and nutritive conditions of life in captivity domesticated animals breed earlier and longer, and are more prolific than their wild ancestors.

FERTILITY

Literature on the fertility of wild gray rats deals chiefly with records of the young found in nests at different seasons

TABLE 8

Litter production and average litter size in different generations of captive gray rats

GENERA-TION OF BREEDING FEMALES	NUMBER OF BREEDING FEMALES	TOTAL NUMBER OF LITTERS	AVERAGE NUMBER OF LITTERS PER FEMALE	TOTAL NUMBER OF YOUNG	AVERAGE NUMBER OF YOUNG PER FEMALE	AVERAGE NUMBER OF YOUNG PER LITTER
10	54	260	4.81	1538	29.6	5.91
11	55	305	5.55	1827	33.2	5.99
12	50	312	6.24	1877	37.5	6.01
13	55	333	6.05	1906	34.7	5.72
14	56	384	6.86	2225	39.7	5.79
15	57	398	6.98	2343	41.1	5.89
16	55	492	8.95	3109	56.5	6.32
17	53	486	9.17	2986	56.3	6.14
18	58	584	10.07	3746	64.6	6.41
19	54	550	10.18	3472	64.3	6.31
20	53	437	8.25	2719	51.3	6.22
21	61	556	9.11	3329	54.5	5.98
22	58	503	8.67	2971	51.2	5.91
23	59	479	8.12	2905	49.2	6.06
24	60	555	9.25	3387	56.4	6.10
25	51	512	10.04	3232	63.3	6.31
10–25	887	7146	8.06	43572	49.1	6.10
1–25	1304	8685	6.66	53077	40.7	6.11

of the year, and with data for the number of fetuses in gravid females of unknown ages. Estimations regarding litter production and litter size differ greatly. According to various accounts (Zuschlag, '03; Lantz, '10; Miller, '11; Eaton and Stirrett, '28), wild females produce an average of from three to eight litters a year: litter size has been calculated as averaging from six to ten young (Crampe, 1884; Zuschlag, '03; Lloyd, '09; Lantz, '10; Miller, '11).

Females in the first nine generations of captive grays cast, on the average, 3.69 litters each, and the average litter contained 6.17 young (King and Donaldson, '29: table 7). Data for litter production and for litter size in later generations are given in table 8.

The average number of litters produced by females of the tenth generation (4.81) was greater than that of females in any preceding generation. Subsequently, litter production increased slowly until the nineteenth generation, when females cast an average of 10.18 litters (table 8). The slight decline in litter production during later generations can be attributed mainly to various changes in the location of the colony which
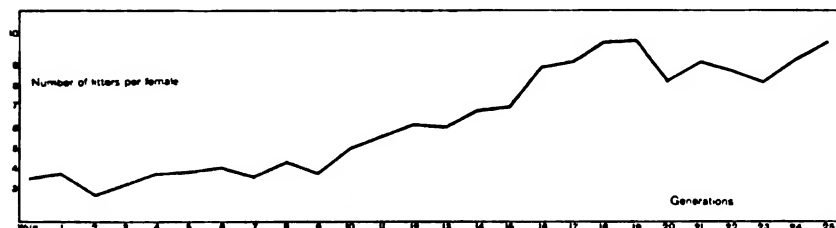
Fig. 9 Average litter production of females in different generations.

adversely affected fertility as well as the body growth of the rats. With this rise and fall of litter production there was a corresponding change in the number of young cast, consequently average litter size in any generation did not differ materially from the mean (6.10) for the entire series (table 8). The general trend in litter production during the course of this investigation is depicted in figure 9.

STERILITY

The infertility of wild rats brought to the colony at the beginning of this investigation and the high percentage of sterility in females of the early generations of this strain were discussed in the former report.

Later work has tended to support the hypothesis, previously advanced, that sterility in wild rats when first captured,

as well as that in individuals of early generations born in captivity, was due to disturbances of the nervous system induced by fear and confinement within a limited space. The nervous tension thus produced probably influenced the activity of many organs of the body, especially those concerned with secretion, and thus indirectly affected the reproductive organs so that they were unable to function normally. As the nervous tension was lessened, after a relatively short period of captivity, the rats began to breed at a more normal rate.

During the first eight generations sterility in females decreased from 37.29 per cent to 5.88 per cent. It rose to 18.64 per cent at the ninth generation, because of the prevalence of ovarian tumors and infections of the genital tract at that time. Only five of the 161 females reared in the tenth to the twelfth generation did not breed, and in these cases sterility was caused by diseases that affected the reproductive organs. In later generations all females reared were fertile. By this time the rats had lost their fear of man and were so well adjusted to their new environment that restriction to reproduction induced by removal from their natural habitat had disappeared.

LITTER SIZE

Data for litter size in the second to the twenty-sixth generation are given in table 9. Litters of the first generation are not included in this table because of the probability that the data obtained did not cover the entire litter output of the wild females that bred while in captivity. Data for five generations are grouped together to facilitate their analysis.

The range in litter size was from one to fifteen, with the mean at six. In each of the first three groups of table 9, over half of the litters were of medium size, containing from five to seven young. The last two groups comprise a relatively smaller number of medium sized litters and more litters below or above this magnitude. This difference in the distribution of the various sized litters in the groups was due, doubtless, to the extension of the reproductive period and the increased fertility of females in the later generations (table 8).

TABLE 9

Litter size, by generation groups, in the second to the twenty-sixth generations of captive gray rats

GENERATION GROUPS	LITTER SIZE															TOTAL NUMBER OF LITTERS
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
2- 6	1	24	57	89	111	161	144	88	55	23	9	2	2			766
7-11	2	17	82	102	167	230	166	138	63	24	14	5		1	1	1012
12-16	17	71	143	241	266	370	238	193	104	62	19	4	3	1		1732
17-21	20	117	203	286	310	442	374	336	226	148	53	26	5	2	1	2549
22-26	29	114	240	299	397	463	362	276	229	116	45	27	6	2	1	2605
2-26	69	343	725	1017	1251	1666	1284	1031	677	373	140	64	16	6	2	8664

When the reproductive period is long, litters cast at its beginning and near its close tend to be small. Increased fertility leads not only to the production of a greater number of litters, but also, in most cases, to the casting of larger litters during the height of reproductive activity.

The summary of data in table 9 is shown by the graph in figure 10, which indicates a very symmetrical distribution of the various litter groups around the modal point.

Litter size in captive grays, as in other varieties of rats, is not affected by the season of the year in which birth
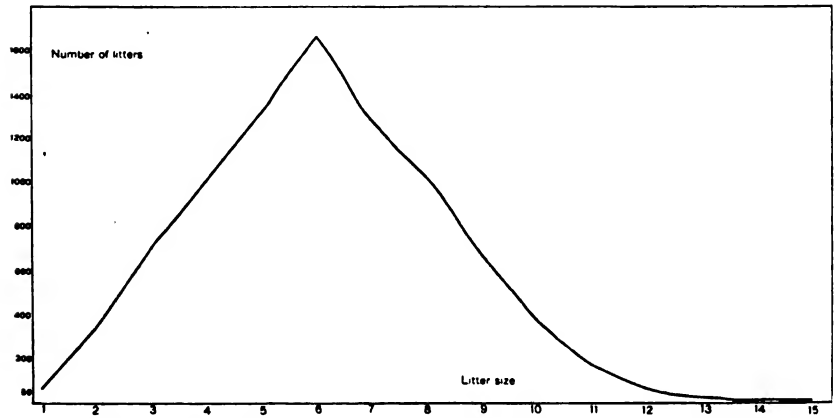
Fig. 10   Litter size in the second to the twenty-sixth generation.

occurs (King, '27): In this respect, findings for the rat accord with those for the mouse (Parkes, '26 b), the pig (Machens, '15; Carmichael and Rice, '20; Parkes, '26 a) and the dog (Dighton, '22).

Since a previous investigation has shown that the age of the mother has a pronounced effect on both litter production and litter size in rats (King, '16 a), a brief analysis of the series of data for gray rats with reference to this point is given here. Data for litters cast in the second to the twenty-sixth generation, arranged according to the age of the mothers at the time of parturition, are shown in table 10.

As indicated in table 10, comparatively few litters were cast when females were not more than 3 months of age. Although the sex organs are capable of functioning at this age, the bodies of the females are not fully grown and so less able to nourish embryos than when they are more mature. The number of litters cast increased with the age of the mothers to the period of greatest productiveness, which came

TABLE 10

Litter production and litter size with their coefficients of variation in the second to the twenty-sixth generation of captive gray rats. Data arranged according to the age of the mothers at the time of parturition

MOTHERS' AGE IN MONTHS	TOTAL NUMBER OF LITTERS	AVERAGE SIZE OF LITTERS	COEFFICIENTS OF VARIATION FOR LITTER SIZE
3	238	5.42	35.0±1.08
4	437	5.98	34.4±0.78
5	518	6.29	31.9±0.67
6	566	6.33	32.5±0.65
7	620	6.37	33.5±0.64
8	608	6.55	33.2±0.64
9	608	6.38	34.0±0.66
10	596	6.40	34.6±0.67
11	559	6.06	36.6±0.74
12	533	6.14	37.9±0.78
13	535	6.37	37.6±0.77
14	493	6.00	38.8±0.83
15	512	6.10	37.5±0.79
16	468	5.93	39.6±0.87
17	419	5.98	39.9±0.86
18	419	5.72	41.6±0.97
19	306	5.29	46.6±1.26
20	130	5.20	49.2±2.05
21+	99	5.18	41.8±1.99
	8664	6.10	36.2±0.19

when females were 7 months old, and then declined as the intervals between litters became longer. Few litters were cast after females reached the age of 20 months. Autopsies made on very old females some times disclosed embryos in the uterus that were being resorbed, probably because senility changes in the uterus so interfered with the nutrition of the young that they died at an early period of development.

The average size of the litters increased until the mothers were 8 months old, when the maximum (6.55) was attained (table 10). Subsequently litter size decreased slowly, and dropped to its lowest point (5.18) at the end of the series. The rise and fall in average litter size as the age of the mother advanced is shown by the graph in figure 11, constructed from data in table 10.

A change in litter size as the age of the mother advances has been reported in other strains of rats (Crampe, 1883; Slonaker and Card, '23; King, '16 a, '24), as well as in other polytocous mammals: guinea pig (Minot, 1891; Wright, '22), rabbit (Hammond, '14), and the pig (Hammond, '14; Carmichael and Rice, '20; Keith, '30).

This series of data for gray rats, covering the litter production of a large group of females, gives 6.1 as the average
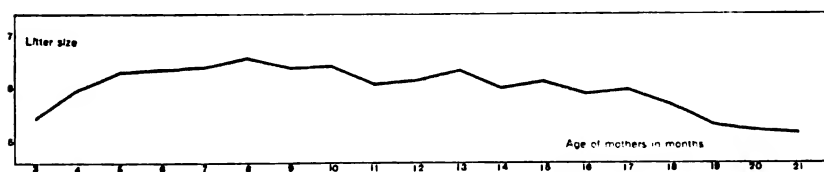
Fig. 11 Age of the mothers and litter size in the young.

for litter size. This average is the same as that found in 815 litters cast by albino females (King, '24: table 1). The norm for litter size in albinos is usually given as seven. This estimation is based, for the most part, on random samplings of litters produced in colonies where females were bred only during the most active period of reproductive life and is therefore too high, since it does not include the litters that might have been cast in the beginning and near the end of the normal reproductive period. The latter litters, as this and other investigations have shown, are usually small as compared with litters produced when females are 5 to 9 months old.

Variation in litter size was high regardless of the age of the mothers, as is indicated by series of coefficients of variation given in table 10. Variation changed considerably, however, as the age of the mothers advanced. It was slightly

below the mean in litters cast when females were very young, and significantly lower when females were at the height of reproductive activity at 5 to 9 months of age, deviations being more than three times their probable errors. When fertility began to decline, variation in litter size increased gradually, reaching its highest point in litters produced near the end of reproductive life. These changes in the variation of litter size as the age of the mothers advanced are very similar to those reported by Little ('33) in a strain of inbred mice.

The age of the female, seemingly, is a factor that profoundly influences her entire reproductive life. It determines to a great extent not only the beginning and the end of her sexual activity, but also produces cyclic changes in litter production, in litter size, and in the variation of litter size. It may prove, also, to have an effect on the functioning of the germ cells, as Little ('33) has suggested is probable.

THE SEX RATIO

Little is known regarding the sex ratio in gray rats living in their natural habitat. The proportion of the sexes found in large numbers of trapped adults gives no information of value on the sex ratio in the newborn, since mortality during early postnatal life may have taken a heavier toll of one sex than of the other.

Two small series of data have been recorded that give the sex proportions in the young of wild females brought into captivity: Miller ('11) found a sex ratio of 82.1 males to 100 females in fifty-one offspring of a caged wild female; the sex ratio in 139 young obtained at the beginning of this investigation from matings of wild rats was 98.6 males to 100 females. This evidence, meager though it is, indicates that a sex ratio below equality may be normal for wild rats. Since these animals are polygamous, a preponderance of females among them would not be detrimental to the survival of the race.

Sex ratios for individuals in the first ten generations of captive grays were given in the former report on this strain, and discussed with reference to various factors that might

have influenced them. Data for sex distribution and for sex ratios in rats of later generations are given in table 11.

The sex ratios in table 11 are discordant, and do not show any definite trend as the generations advanced. In many cases a high ratio in one generation is followed by a relatively low ratio in the succeeding generation, and there are no significant differences between ratios for successive generations

TABLE 11

Sex distribution and sex ratios in the eleventh to the twenty-sixth generation of captive gray rats

GENERATION	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
11	1538	752	786	95.7±3.27
12	1827	946	881	107.4±3.39
13	1877	953	924	103.2±3.21
14	1906	907	999	90.7±2.81
15	2225	1128	1097	102.8±2.94
16	2343	1171	1172	99.9±2.78
17	3109	1561	1548	100.8±2.44
18	2986	1458	1528	95.4±2.36
19	3746	1823	1923	94.3±2.08
20	3472	1716	1756	97.7±2.24
21	2719	1311	1408	93.1±2.42
22	3329	1659	1670	99.3±2.32
23	2971	1495	1476	101.3±2.51
24	2905	1412	1493	94.6±2.37
25	3387	1634	1753	94.5±2.19
26	3232	1572	1660	94.7±2.25
11–26	43572	21498	22074	97.4±0.63
1–10	9505	4680	4825	96.9±1.34
1–26	53077	26178	26899	97.3±0.57

nor between any one ratio and that for the series as a whole. The ratio for individuals in the eleventh to the twenty-sixth generations is but slightly higher than that for the first ten generations, and for the combined series is below equality, being 97.3 males to 100 females.

Figure 12 shows graphically the sex ratios for the first twenty-six generations of captive grays.

The lowest point in the graph of figure 12 is at the second generation, where the sex ratio falls to 81.9 males to 100 females. Thereafter the graph tends to rise, reaching its highest point (107 males to 100 females) at the twelfth generation, then declining gradually and ending in a nearly straight line which is below the level of the mean. A male excess among individuals is indicated at only eight of the twenty-six points in this graph, and there are no changes in it, except possibly the drop near its beginning, indicating that captivity per se affected the ratios in any way.
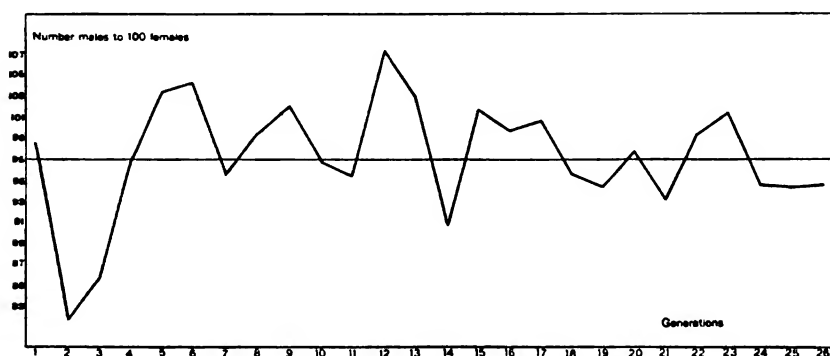
Fig. 12 Sex ratios in different generations.

Data for a series of stock albino rats, comprising the litter output of a group of individuals reared under the same conditions of environment and of nutrition as the early generations of captive grays, give a mean sex ratio of 105.2 ± 2.00 (King, '24). This ratio is significantly higher than that found in gray rats, the difference being 7.9 ± 2.08. The disparity in the sex ratios of these two strains must be due either to errors in recording the data or to differences inherent in the strains, since they cannot be ascribed to unlike conditions of life.

Depletion of litters through destruction of stillborn young by adults in the cage is the most common source of error in litter records for rats. Such errors cannot be avoided, since parturition often occurs at night and some hours may elapse before the young are examined, but they have relatively little

effect on a large series of litter data, since the stillborn form but a small percentage of the young cast, as a rule. In 968 litters of gray rats obtained at birth and known to be complete, the stillborn comprised but 1.2 per cent of the 6295 individuals (King, '35). This finding accords well with that of 1.3 per cent stillbirths in 31,670 albino rats (King, '21). Since only 0.05 per cent stillbirths were recorded through the first twenty-six generations of gray rats, it is possible many stillborn young were devoured by adults in the cage before the litters were examined. In a former paper (King, '21) it was estimated that the normal percentage of stillbirths in any strain of rats reared under favorable conditions of environment and nutrition is probably not greater than 2 per cent. If, therefore, the number of young in the final summary of table 11 is increased to include 796 (1.5 per cent) stillborn young that, conceivably, might have been omitted from the records, with the sexes proportioned to give approximately the ratio among the 268 stillborn young recorded for the strain (111.8 males to 100 females), the data so adjusted would raise the final sex ratio in table 11 from 97.3 to about 97.5 males to 100 females. Obviously the low sex ratio found in gray rats cannot be ascribed to the omission of stillborn young from the records, unless the number of such individuals greatly exceeded 2 per cent of the total and the sex ratio among them was unusually high. There are no valid grounds for either of these assumptions.

In an investigation of the factors controlling fertility in the rabbit, Hammond ('14) found that the number of atrophic fetuses in females of various domesticated breeds greatly exceeded that found in gravid wild females. He suggested that possibly one of the effects of domestication on this animal has been to increase the number of ova shed at each period, and at the same time to reduce the proportion of those which develop. In the gray rat, captivity over a considerable period of time has greatly increased fertility, but has not changed average litter size (table 8). If the number of ova shed at each period has been increased by domestication, then fetal

atrophy has increased also, or average litter size would not remain constant. It is known, from the investigations of Huber ('15) that many rat fetuses die soon after implantation. Such fetuses, as well as those that die at later stages of gestation, are absorbed in situ and not aborted as in higher mammals, therefore the proportion of the sexes among them cannot be determined.

Recent work in genetics has thrown much light on the cause of fetal mortality, by showing that germ cells frequently contain lethal or semi-lethal genes that either kill the embryos carrying them, or so reduce their vitality that they rarely survive. Such deleterious genes have been reported in mice (Little, '19; de Aberle, '27; Hagedoorn and Hagedoorn, '28; Chesley, '35; Kamenoff, '35) and in the rabbit (Hammond, '33). An assumption that germ cells of gray rats contain genes that tend to kill or greatly reduce the vitality of embryos, particularly males, would offer a plausible explanation for the low sex ratio among the newborn.

Since the sex ratio in gray rats is significantly lower than that in albinos, it may be of interest to compare the sex ratios in white races of man with those for colored races, chiefly of negro descent, when these races live side by side under the same topographical and climatic conditions, as shown in table 12. This tables is based on one given by Parkes ('26), augmented by the inclusion of stillbirths in some entries. The probable errors of the sex ratios are given when the data seem to be sufficiently accurate to warrant their calculation. To this table has been appended the sex ratio for captive gray rats (table 11) and that for the series of albino rats maintained under the same environmental and nutritive conditions (King, '24: table 1).

In all groups cited in table 12 the sex ratios for black races are lower than those for white races. When judged by their probable errors, differences between corresponding ratios are significant for the data from Heape ('09) and from Little ('20), but not for data given by Nichols ('07). Jastrzebski's ('19) data, given in round numbers, cannot be considered as

reliable as those from other sources. In these groups, differ-
ences between the ratios are probably not important, except
in the data from the U.S.A. Parkes ('26) states that the
lower sex ratios in black races seem to be a general phenom-
enon that probably has some real basis. He considers it due
to greater prenatal mortality in the blacks, among whom abor-
tions and stillbirths are greater than in white races. The sex
ratio in man, as in other mammals, seems to be one of the
characteristics of the race that depends upon a number of

TABLE 12

Sex ratios in white and in colored races of man

AUTHORITY	LOCALITY	WHITE RACES		COLORED RACES	
		Number of individuals	Number of males to 100 females	Number of individuals	Number of males to 100 females
Heape, '09	Cuba	70684	108.4±0.39	21028	101.1±0.67
Little, '20	New York	1834	118.3±1.71	1418	96.1±1.76
Nichols, '07	District Columbia	71024	107.3±0.54	56034	105.8±0.60
Jastrzebski, '19	Cape Colony	100000	105.4	100000	102.6
	U.S.A.	700000	105.7	12000	100.0
	New York	700000	104.5	10000	101.6
	New Orleans	12000	102.0	4000	98.2
	District Columbia	20000	105.0	9000	100.0

Sex ratios in albino and in gray rats

		ALBINO RATS		GRAY RATS	
King, H. D.	Wistar Inst. colony	4992	105.2±2.00	53077	97.3±0.57

factors, some of which are genetic, others physiological or
environmental. It is not improbable that the genetic consti-
tution of a race may prove to be one of the most important
of the various factors that affect the vitality of fetal young
and so largely determine the sex ratio among the newborn.

The hypothesis that litter size affects the sex ratio in poly-
tocous mammals is one of long standing, and various series of
data have been recorded both for and against it. An arrange-
ment of data for gray rats to indicate the relation between
litter size and the sex ratio is shown in table 13.

Litters containing more than twelve young are included in table 13 merely to complete the records. They are too few to be of value statistically, and the ratios are not in accord with those for other litter groups.

The great majority of litters in this series contained from four to eight young, and the sex ratios for these groups vary little from each other or from the ratio for the series as a whole. In litters of one and in litters containing eleven or twelve young the sex ratios are considerably lower than those

TABLE 13

Litter size and the sex ratio in the second to the twenty-sixth generation of captive gray rats

LITTER SIZE	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
1	69	69	33	36	91.7±14.94
2	343	686	340	346	98.3± 5.06
3	725	2175	1061	1114	95.2± 2.75
4	1017	4068	2027	2041	99.3± 2.09
5	1251	6255	3048	3207	95.0± 1.62
6	1666	9996	4989	5007	99.6± 1.34
7	1284	8988	4437	4551	97.5± 1.38
8	1031	8248	4089	4159	98.3± 1.46
9	677	6093	2974	3119	95.4± 1.65
10	373	3730	1844	1886	97.8± 2.16
11	140	1540	739	801	92.3± 3.17
12	64	768	362	406	89.2± 4.34
13	16	208	112	96	116.7±10.95
14	6	84	42	42	100.0±14.73
15	2	30	12	18	66.6±16.74
1–15	8664	52938	26109	26829	97.3± 0.57

for the other litter groups, but the differences are not important statistically. Data in table 13 do not indicate any definite relation between litter size and the sex ratio in gray rats, and so are in agreement with the findings for many other mammals: albino rat (King and Stotsenburg, '15; mouse (Parkes, '26 b); deer mouse (Sumner, McDaniel and Huestis, '22); guinea pig (Ibsen, '22; Schott and Lambert, '30; Haines, '31) and the pig (Parker and Bullard, '13; Machens, '15; Parkes, '23).

It has been shown that in each of four different strains of rats the sex ratio changed in a similar way as the litter series advanced (King, '24: table 8). This finding seems to indicate that the age of the mother has some influence on the sex ratio in her newborn young. As a further test of this assumption, litter data for gray rats were arranged according to the

TABLE 14

Sex distribution and sex ratios in the second to the twenty-sixth generation of captive gray rats. Data arranged according to the age of the mothers at the time of parturition

MOTHER'S AGE IN MONTHS	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
3	1291	661	630	104.9±3.94
4	2613	1322	1291	102.4±2.70
5	3262	1633	1629	100.2±2.37
6	3582	1758	1824	96.4±2.17
7	3955	1965	1990	98.7±2.12
8	3984	1977	2007	98.5±2.10
9	3881	1923	1958	98.2±2.11
10	3815	1885	1930	97.7±2.13
11	3389	1716	1673	102.6±2.37
12	3277	1647	1630	101.0±2.38
13	3309	1641	1668	98.4±2.25
14	2960	1407	1553	96.6±2.26
15	3126	1542	1584	97.4±2.34
16	2777	1331	1446	92.1±2.36
17	2510	1226	1284	95.5±2.57
18	2397	1169	1228	95.1±2.62
19	1620	751	869	86.4±2.90
20	677	314	363	86.5±4.49
21+	513	241	272	88.6±5.28
	52938	26109	26829	97.3±0.57

age of the mothers at the time of parturition, and the sex ratios calculated. The series of ratios thus obtained is given in table 14.

Males outnumbered females in litters cast when mothers were from 3 to 5 months of age, and also in litters born when the mothers were 11 and 12 months old. At all other age periods of the mothers litters contained an excess of females,

this excess being most marked in offspring produced near the close of reproductive life (table 14). The change in the sex ratio as the age of the mother advanced is shown by the graph in figure 13, which was constructed from data in table 14.

Beginning relatively high, the graph in figure 13 falls gradually for a time, rises again at the middle of its course, and then declines until its end. This cyclic change in the sex ratio with the advancing age of the mothers is very similar to that found in the four strains of rats, mentioned above, where data were arranged by parity. The accord in these various series of ratios can hardly be a matter of chance, although in none of them are differences between the ratios in various groups important when judged solely by their probable errors.
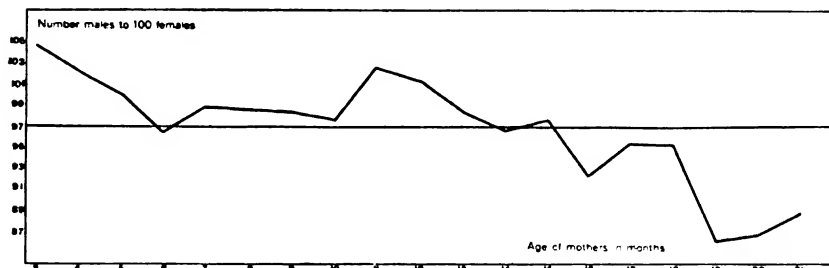
Fig. 13   Age of the mothers and the sex ratio in the young.

Few of the studies on lower mammals in which data have been analyzed with reference to the relation between the age of the mothers and the sex ratios in the young have given consistent results, since the data were obtained, for the most part, by the method of 'random sampling,' and therefore do not cover the complete breeding records for a given group of females. Investigations on the guinea pig, made by Ibsen ('22), showed a difference of 6.06 times the probable error between the sex ratio in young from mothers 8 months old and that in offspring of females 15 months of age. In later data obtained from this animal, Ibsen and Burhoe ('28) found no relation between the age of the mother and the sex ratio in the young, "thus furnishing proof that results biometrically

significant do not always receive biological verification.'' The more extensive series of data for the guinea pig, given by Kröning ('34), shows that the sex ratio tends to decrease as the age of the mother advances. This investigator explains his findings in accordance with the old Hofacker-Sadler hypothesis that the sex ratio in the young is influenced by the relative age of the parents.

In the large and carefully compiled series of data for Flemish and for Swedish breeds of cattle, summarized by Johansson ('32: table 6), the general trend of the sex ratio in each series is cyclic, and much like that in table 14, except that the ratios are relatively low in the early born young. In Johansson's series, as in these for gray rats, differences between ratios are not important statistically, therefore the findings can be considered merely as suggestive.

Literature contains numerous references to investigations on the sex ratio in various races of man, based chiefly on data from governmental registrations of vital statistics and on geneological or hospital records. In many of these studies the results accord in showing that the sex ratio is very high for first births and then tends to fall as the age of the mother advances (Düsing, 1884; Rosenfeld, '00; Newcomb, '04; Punnett, '04; Lewis and Lewis, '06; Siegel, '17; Parkes, '24, etc.). The usual explanation given for this change in the sex ratio is that there is an increase in the prenatal mortality of males as the mothers grow older, but why this increased mortality occurs is not known. Age changes in body metabolism, in the structure of the uterus or in hormone secretions affecting reproductive processes probably affect the vitality of fetal young, and may tend to eliminate more males than females. However, until more light has been shed on the effect of such factors, it will be useless to speculate as to the cause for the change in the sex ratio of the young as the age of the mother advances.

MORTALITY

No attempts were made to determine the potential duration of life in gray rats, since it seemed more important to ascertain the condition of various body organs before senility had altered them.

In all generations, the study of life processes was terminated when rats reached the age of 20 months, and a stated number of individuals given to Doctor Donaldson for a determination of organ weights. Exceptions were made in the case of females that were unusually vigorous and had cast litters when they were over 19 months of age. Such females were housed with young males, and not killed until their reproductive activity had ended in order that the series of litter data might be complete.

Mortality at birth was very low in all generations, and only 268 stillbirths were recorded among the 53,077 young born in the first twenty-six generations. Mortality among the young during the suckling period decreased considerably after the tenth generation. By this time females were so well adjusted to conditions of captivity that they rarely destroyed their offspring returned to the nest after they had been removed for examination, and nests were less frequently disturbed by other inmates of the cage. Offspring were destroyed or neglected only when the mother was in poor physical condition, or when one or more of her nipples had been injured by the young when suckling.

Table 15 gives mortality data for the eleventh to the twenty-fifth generation, with a summary of the records for the entire series grouped to indicate the general trend in mortality rate as the generations advanced.

In later generations the mortality rate in both sexes was low during the first year of life, the relatively high percentage (11.9) in the twenty-third generation being due to the accidental killing of four young females (table 15). Mortality increased greatly after the rats were a year old, and only 79.9 per cent of males and 76.6 per cent of females lived to the age of 20 months.

TABLE 15

Mortality in various generations of captive gray rats

GENERATIONS	MALES					FEMALES				
	Number of individuals	Number living at 12 months	Per cent mortality at 12 months	Number living at 20 months	Per cent mortality at 20 months	Number of individuals	Number living at 12 months	Number living at 20 months	Per cent mortality at 12 months	Per cent mortality at 20 months
11	54	52	3.7	42	22.2	57	56	45	1.8	21.1
12	52	49	5.8	37	28.8	52	50	39	3.8	25.0
13	55	52	5.5	45	18.2	56	53	39	5.3	30.4
14	55	52	5.5	43	21.8	56	55	41	1.8	26.8
15	53	48	9.4	42	20.8	57	55	39	3.5	31.6
16	61	59	3.3	50	18.0	55	55	44	0.0	20.0
17	55	50	9.1	44	20.0	55	52	42	5.5	23.6
18	57	52	8.8	43	24.6	58	58	49	0.0	15.9
19	54	53	1.9	48	11.1	54	54	41	0.0	24.1
20	56	55	1.8	46	18.2	53	51	39	3.7	26.4
21	54	51	5.6	42	22.2	61	59	47	3.3	22.9
22	57	57	0.0	47	17.5	58	56	46	3.4	20.7
23	60	58	3.3	49	18.3	59	52	41	11.9	30.5
24	59	56	5.1	44	25.4	60	60	50	0.0	16.6
25	50	50	0.0	43	14.0	51	50	43	1.9	15.7
1–10	521	502	3.6	321	38.4	542	521	339	3.9	37.4
11–15	269	253	5.5	209	22.3	278	269	203	3.3	26.9
16–20	283	269	4.9	231	18.4	275	270	215	1.8	21.8
21–25	280	272	2.8	225	19.7	289	277	227	4.2	21.5
1–25	1353	1296	4.2	986	27.1	1384	1337	984	3.4	28.8

The summary of data in table 15 shows no general trend in the mortality rate at 12 months in either sex. In males, the lowest rate (2.8 per cent) comes for individuals in the last group, while in females the rate for this group is high (4.2 per cent) only because of accidental deaths. In both sexes, however, the mortality at 20 months tended to decrease as the generations advanced. In males of the last group, mortality was 18.7 per cent below that in the first group; in females it was 15.9 per cent less. Conditions of life in captivity thus tended to prolong the average life span of the individuals, possibly because they received more adequate nutrition and were protected from various diseases prevalent among wild rats living in their natural habitat.

The main causes of death among older individuals were the lung infection, commonly called 'pneumonia,' and tumors of various kinds. Pneumonia rarely attacks rats until they are a year old, and males seem more susceptible to it than females. Autopsies made on rats 20 months old have shown very few individuals that were entirely free of this disease. Tumors occur mainly in females, and are usually found in the ovaries or mammary glands. The latter growths, when small, can be removed easily, and rarely return. Cancer has appeared but rarely in this strain, as it has been found only in one male and in three females. Gray rats are very resistant, apparently, to the middle-ear infection commonly found in other strains of rats. Only eight of 2737 individuals reared for study developed this disease, and in two of these cases it could be attributed to the fact that gray females were serving as 'foster mothers' to young from an albino strain in which many individuals were affected.

BEHAVIOR

After 14 years of life in captivity the behavior of gray rats had changed so greatly that many of the obnoxious traits exhibited by the early descendants of feral animals had disappeared. These changes in behavior occurred so slowly, however, that there was no noticeable difference in the conduct

of individuals in any two succeeding generations, although a comparison of the behavior of rats in early and in late generations indicates clearly the striking modifications that occurred.

A high nervous tension and extreme fear of man was shown by all rats in early generations. They ran wildly about the cage even at the approach of colony workers to whom they were accustomed, and constantly gnawed the wire netting and other parts of the cage in their efforts to escape from confinement. When the cage door was opened the rats often jumped directly at the face of the person who thus seemed to offer them a way to freedom. An escaped rat, when captured, showed marked evidence of fear, trembling and clicking its teeth, and sometimes on being returned to the cage lay inert for a few moments and then died. At this time considerable difficulty was experienced in rearing the young. If a nest was disturbed at or soon after the birth of a litter, the mother usually destroyed her offspring. If the members of a litter were removed from the nest, even when they were several days old, only the exceptional females would care for them on their return. Many litters, therefore, had to be reared by foster mothers from other strains.

After the rats had become adjusted to new conditions of life, their fear of man and nervous tension decreased greatly. A rat that escaped from the cage displayed little resistance on being captured, and its behavior was normal when it rejoined the family group. As a rule, rats remained quiet when the cage door was opened, even when they were being inspected by strangers. Many of them came to the front of the cage at the approach of the feeding truck, and would take food offered through the wire netting of the door. Females no longer resented inspection of their offspring, and took excellent care of them. If newborn young were removed from the nest for examination, they would be cared for on their return. Many females would rear litters from other strains if they were barred from the nest until the young had acquired the nest odor. However, alien young were always killed if the

female had access to them as soon as they had replaced her own offspring.

Two of the outstanding traits exhibited by individuals in early generations still persisted at the twenty-fifth generation. Adults continued to show pronounced antipathy to individuals from other litters placed in their cage, and promptly attacked and usually killed them unless special precautions were taken, as described in the previous report. Even these devices were not always effective. This treatment of strangers seems to indicate a retention of the primitive instinct to protect the nest from all entrants not members of the family group. The 'killer' instinct, discussed in a previous section, has been shown by gray rats of all generations, especially by large males. This trait is doubtless of advantage to the species, since the elimination of smaller and weaker individuals insures that the largest and most vigorous animals will be the progenitors of the succeeding generation. In late generations it was no longer necessary, though often expedient, to stupefy rats with ether when they were removed from the cage for weighing, as they could be picked up by the tail with long forceps but not with bare hands.

As this investigation was designed to study the effects of captivity on gray rats, no attempts were made to tame any of the rats used in this work so that they could be handled as are the rats of various strains maintained for general laboratory purposes. While captivity over many years had accustomed these rats to receiving their food from man and to his presence, their innate fear of him did not decrease to the point where they would willingly submit to being held by bare hands. Gray rats can be tamed more easily, perhaps, than any other feral animals, if one has the required patience and understanding of their temperament. Two young rats from the third generation were taken to another colony by Miss Ruth Meeser, of The Wistar Institute, and rendered so tame that they showed no vicious traits when adult, although they were always nervous in the presence of strangers and could be handled only by Miss Meeser. More recently, a number of young gray rats were given to Miss Meeser for taming,

and she succeeded in making them quite as gentle as are
stock albinos. These tamed rats were then used in experi-
ments requiring daily injections of solutions through the ab-
dominal wall. They submitted to these injections as readily
as do albinos, even though they were held by bare hands.
Gray rats, therefore, can now be used for all forms of experi-
mental work. Large, vigorous, and seemingly little susceptible
to certain diseases prevalent in other strains of rats, they
should be valuable for laboratory work in which it seems
desirable to use individuals from a pure strain of known
ancestry.

MUTATIONS

Several mutations affecting the color or the structure of the
hair appeared in captive gray rats during the period covered
by this report. As only a brief report of these mutations has
been published (King, '32), a more detailed account is given
here, including genetic studies made to determine the mode
of inheritance of certain types.

One of the six wild gray females (2) used as foundation
stock for the colony had a small spot of white hair on the
ventral surface of the body between the forelimbs, similar in
size and position to the spot shown in figure 14. This female
was mated with a wild male (2), presumably a brother since
the two rats were trapped at the same time and appeared to
be the same age. From the matings of this pair of rats
twenty-nine young were obtained, of which seventeen had a
patch of white hair on the ventral surface varying in size
from a mere dot to a spot no larger than that in figure 14.
Experiments were then begun to determine whether the
amount of white in coat could be increased by selective breed-
ing. For two generations rats having white spots were inbred,
brother and sister. Subsequent matings were made between
individuals with the greatest amount of white in their coats,
regardless of their relationship.

During early generations there was but little increase in
the size of the white area, but in the sixth generation indi-
viduals appeared with irregularly shaped patches of white

covering the space between the forelimbs (fig. 15). Later the
white was extended, and in some cases a second white spot
appeared (fig. 16). Matings of these rats produced some off-
spring with a long streak of white in the midventral region
of the body (fig. 17). In the fifteenth generation a litter was
obtained in which three of the individuals, one male and two
females, were typical 'Irish' rats in that the four feet, the
tip of the tail, and the ventral surface were covered with
white hair (fig. 18). Presumably at this point the self factor
(H) changed to its allelemorph (h¹), a reverse change to that
found in experiments made by Castle and Phillips ('14) with
hooded rats where the hooded factor (h) changed to h¹. When
'Irish' rats were inbred they produced some offspring like
themselves and others in which the white area was extended
up the sides of the body (fig. 19). Descendants of the latter
rats had coat patterns showing but slight variations from that
in figure 20. Gray hooded rats were kept in the colony for
some 3 years, but no further attempts were made to increase
the amount of white in the coat through selective breeding.
Matings of these rats with black hooded rats from a strain
that had bred true to type for many years produced only gray
hooded young, indicating that the hooded pattern in the two
strains was due to the same gene mutation.

Some years ago Castle and Phillips ('14) carried out an
extensive series of experiments in order to study the effects
of selection on the coat pattern of black hooded (piebald) rats.
These experiments, which were continued through twenty
generations, were divided into two series; in one series selec-
tion was made in a plus direction, and in the other series in
a minus direction. At the end of the experiments individuals
in the plus series were black except for a small area of white
on the ventral surface, similar in its position to the spot shown
in figure 14. In individuals of the minus series black was
restricted to the head region. Castle's ('19) final conclusions
regarding the results of this work were that alterations in the
coat pattern were not due to changes in the gene for the
hooded pattern, but to residual heredity. Selection had uti-
lized the minor genetic changes that are occurring continually

to "move the racial mode and mean either in a plus or in a minus direction without encountering impassible limits short of an all white or an all black condition" (Castle, '31).

In light of Castle's work, it seems probable that a hooded mutation occurred at some period in the ancestry of the wild rats from which gray hooded rats were derived by selective breeding. Evidently breeding under natural conditions, and the action of plus modifiers, had kept recessive the hooded pattern in the wild stock, but not effaced the gene for spotting which manifested its presence only in the coat of the female, as the male with which this female was mated had no white in his hair. By selective breeding through a number of generations plus modifiers were gradually eliminated, or rendered inactive, and the white area increased until the typical hooded pattern was restored.

Aside from the hood strain, developed among the descendants of one pair of wild rats, captive grays bred true to type, as far as known, for a period of 7 years, during which time over 10,000 young were born and many hundreds of them reared to an age when coat characteristics were well defined.

In the eleventh generation one of four females, mated to a brother, cast a total of forty-five young of which eleven died at or a few days after birth so their coat color could not be determined. The remaining thirty-four individuals comprised twenty-four normal grays and ten mutant blacks. The first mutant, a male, appeared in a litter of four, born when the mother was 15 months old. Although sisters of the female that cast black young were mated to the same sire, no mutants appeared among their progeny, nor has this mutation appeared a second time in the strain. These mutants were a very intense black on the dorsal side of the body, and a dark slate color on the ventral side. There was no change in their color for a period of about 4 years. Then individuals appeared in which white hairs were scattered through the coat and among the vibrissae. These white hairs were especially numerous on the sides of the body, and in some cases were found in groups of three to six. Possibly a 'silver' mutation

occurred in these rats, similar to that found in the mouse (Dunn and Thigpen, '30), but no genetic tests of this assumption were made. Later the hair in some individuals had a distinctly brownish tinge, but inbreeding through several generations did not change the coat color to that of the non-agouti brown or chocolate rat.

Routine examination of newborn litters of the thirteenth generation disclosed three individuals in which the eye color seemed somewhat lighter than that normal for gray rats. Rearing these rats, which belonged in a litter cast when the mother was 16 months old, revealed a second mutation in the strain, ruby-eyed dilute. Two more of these mutants appeared in a later litter from the same parents, making a total of five mutants in thirty-four young. Normal young from these litters were reared and used for breeding. As expected, a number of ruby-eyed dilutes appeared among their descendants in later generations. Matings of these mutants with ruby-eyed descendants of the first rats of this type, which were discovered by Whiting (Whiting and King, '18), gave only ruby-eyed dilute young. These two mutations from unrelated gray stocks were due, therefore, to the same recessive gene which dilutes pigmentation in the eyes as well as in the coat.

A third mutation, albinism, was discovered in two rats of the fourteenth generation through the color of the eyes at birth. These mutants were members of the fifth litter cast when the mother was 14 months old. There were no other mutants among her forty-five offspring. Sisters of this female mated to other males produced only gray young, but albinos were found among their descendants during several succeeding generations.

The next mutation found, curly (Cu), was a type not previously recorded in rats, although subsequently two mutations, phenotypically similar but genetically different, were discovered; kinky (k) by Feldman ('35), and curly₂ (Cu₂) by Gregory and Blunn ('36). Curly is a dominant mutation that affects hair structure, not its color. It eliminates or greatly

reduces the size of the guard hairs with the exception of a few around the head, and causes a bend or twist in all hairs of the body including the vibrissae. Because of the modification of the vibrissae, this mutation can be detected in very young rats. The characteristic curl of the hair becomes very pronounced when the rats are 2 weeks old (fig. 21), and the vibrissae are then spiral or bent inward and hooked at the ends (fig. 22). When the rats are about 25 days old the curl begins to disappear and soon the coat looks much like that in normal rats, although it has a soft downy appearance because of the absence of guard hairs (fig. 23). This change in the coat is due probably, as Feldman ('35) has suggested, to the fact that the vigorous pelages of young adults are able to overcome in a large measure the destructive effects of the gene.

The curl reappears in the hair of the mid-dorsal surface when the mutants are about 7 months old (fig. 24), and in the course of 2 or 3 months has extended over the entire coat. This mutation shows its most pronounced effects in old rats where the entire dorsal surface is covered with very curly hair (fig. 25). On the ventral the longer hairs tend to break off or to disappear in various places, thus showing patches of skin between ridges of curly hair (fig. 26).

A histological examination of sections of the skin of curly mutants of various ages disclosed no apparent abnormality in the hair follicles. The wavy appearance of the coat seems to be due to the fact that at intervals along the shaft the cortical layer of the hair becomes very thin, and a bend or a twist occurs at these points.

This mutation was first discovered in a male of the seventeenth generation, born when the mother was 6 months old. Early changes in the pelage had not been noted, but the unusual appearance of the coat when the curl had returned after the 'latent' period aroused suspicion that a new mutant type had appeared. Fortunately, this male had sired a litter which had been saved for study. The four females in this litter had normal coats; the two males were curly. Nine litters were obtained from the matings of these curly males with five normal gray females, two of which were their sisters. These

litters contained sixty-two young of which eight were still-born. Of the fifty-four living young, twenty-five were curly and twenty-nine were normal. The proportion of normal and mutant young was thus near the 1 to 1 ratio to be expected when one parent is a heterozygous dominant. Normal rats from these litters, when inbred, produced only normal young. Breeding the curly rats inter se gave eighty-nine litters with a total of 544 young of which twenty-eight were stillborn. As many of the young died shortly after birth, coat character-istics could be determined for only 266 individuals, of which 181 were curly and 85 normal. Among the curly rats there were 82 males and 99 females, so the mutation is autosomal and not sex linked. The number of curly rats in these litters was below the number (199) to be expected, but doubtless there was an excess of curly rats among the individuals that died young, since these mutants showed the same lack of vitality and vigor that has been found in other mammals in which morphological variations have appeared.

From the breeding tests described above it is evident that curly is a mendelian dominant differing from smooth coat by a single gene. Rats heterozygous for curly show the mutant characteristics in a somewhat less degree than do homozy-gotes. In them the period when the coat is barely distinguish-able from that in normal grays is longer, and in older animals the wave in the hair is less pronounced than it is in homozy-gotes of like age.

A strain of curly mutants was developed for study and for experimental use. In the early generations curly rats were much inferior to normal grays in fertility and in vigor, and mortality among the young was excessive. After sufficient mutants were available to make possible a rigid selection of breeding stock there was a marked increase in fertility, mor-tality among the young decreased, and adult individuals com-pared favorably with normal grays in size and vigor.

Structural modifications that produce curly or wavy hair occur in many mammals, including man. When the hair be-comes very curly or wooly, the gene responsible has been

found to be a dominant, as is the case in various ulotrichous races of man, in European races of white stock (Mohr, '32; Schokking, '34), in karakul sheep (Macalik, '32; Tänzer, '32), in swine (Rhoad, '34), in caracul mice (Carnochan, '37; Dunn, '37), as well as in curly and curly$_2$ rats (King, '32; Gregory and Blunn, '36). On the other hand, if the wave in the hair is slight or tends to disappear with advancing age, the mutation, seemingly, is always a recessive, as in the rex rabbit (Castle, '29), kinky rats (Feldman, '35), and wavy mice (Crew, '33; Keeler, '35).

After the appearance of the curly mutation it was decided that an accumulation of mutant genes in the stock was undesirable, since such genes might possibly affect growth or other life processes in individuals carrying them. Therefore, from the eighteenth generation, all litters reared were offspring of parents that, according to the breeding records, did not carry any of the mutant genes known to be present in the strain. To facilitate the elimination of such genes, individuals in all litters reared in the twenty-first to the twenty-fifth generations were inbred brother and sister.

No new mutations appeared in the strain until the twenty-second generation, when several rats, offspring of brother and sister matings, were found to have more brown pigment in their coats than is present in typical gray rats. Inbreeding these rats gave young of which approximately one-fourth were brown agouti, or cinnamon. This mutation had not been reported previously in rats, although it was known in other rodents (Castle, '31). Breeding tests showed that cinnamon is a simple mendelian recessive, in which brown pigment replaces black throughout the coat (King, '32). Crossing cinnamon rats (AbCH) with pure blacks (aBCH) gave only normal gray young. Inbreeding the F$_1$'s from this cross gave non-agouti brown or chocolate rats (abCH).

Captive grays have bred true to type since the appearance of the cinnamon mutants. However, two other variations from the normal type have been found in mutant strains, aside from the possible 'silver' mutation in black rats. The

waltzing character (w), long known in mice (Yerkes, '07) and reported in other species of rats by Bonhote ('12) and by Hagedoorn and Hagedoorn ('22), appeared in the fourth generation of mutant albinos (King, '36). Another new mutation, which has been designated 'stub' (st), was found in the eighth generation of cinnamon rats. Stub is a simple mendelian recessive that has a very deleterious effect when in a homozygous condition. It reduces the number of tail vertebrae, frequently produces malformations in the posterior region of the body, markedly affects the viability of the young and greatly retards body growth in individuals that survive. A full account of this mutation will be published elsewhere.

There is little in the breeding history of gray rats to support an assumption that genes for these various mutations were present in the foundation stock and remained latent until chance matings brought them to light. The hooded gene, as shown above, was carried by one pair of wild rats (female 2: male 2), so this mutation requires no further comment. All other feral rats that bred in captivity produced only normal gray young, as far as known. The F_1 generation comprised twenty-one litters containing 139 young, of which 106 were raised to maturity. These litters, as well as those born in later generations, were carefully examined at or soon after birth, in order to detect any structural anomalies or any variations in coat color that might be disclosed through changes in eye pigmentation, such as albinism or ruby-eyed dilute. Since black rats cannot be distinguished from grays until the pelage develops, this mutation might have escaped detection in early generations.

Although most of the offspring of wild rats were reared by albino foster mothers, all of them were as savage and as difficult to care for as were their parents. Tests made indicated that individuals that had been reared apart would not always live together amicably when adult. Therefore, to avoid the loss of valuable animals, it was deemed advisable to keep members of the same litter together, whenever it was possible to do so. All F_1 individuals were inbred, brother and sister,

with the exceptions noted below. Aside from the rats carrying the hooded gene, no mutants were found among the 857 individuals comprising the F_2 generation. Any simple recessive color genes present in the foundation stock would have manifested their presence in the second generation, unless they were carried by female 1 and her mate, whose offspring could not be inbred.

It may be of interest to record some data regarding the first pair of wild rats, since their descendants have been dominant in the strain from early generations. Female 1 was brought to the laboratory in February, 1919, having been trapped in an abandoned stable in the outskirts of Philadelphia. She was approximately 6 months of age when captured, and was conspicuous from arrival because of her ferocity. She killed the first and second male with which she was housed, although both were larger than herself. The third attempt to mate her was successful, the male being a young adult captured in March, 1919. Their progeny comprised twenty-five young with the unusual sex ratio of three males and twenty-two females. All of these offspring were reared, with the exception of one female which died when about 2 weeks old, and all were normal grays. Eleven females in these litters were housed with their three brothers for several months, but no young were obtained. Ten females were mated with excess males from litters of the third pair of wild rats. As inbreeding the other offspring of wild female 3 and her mate gave fifty-eight normal gray young, this pair of wild rats did not carry genes for any of the recessive color mutations that later appeared in the strain.

Rats of the second generation were far better adjusted to conditions of captivity than were their parents. Individuals of one litter could be housed with those from other parents, with few casualties resulting. From this time until the twenty-first generation, few matings were made between rats that were very closely related. The young obtained by mating female offspring of the first pair of wild rats with males from litters of the third pair were so noticeably larger and more

vigorous than other rats in the F_2 generation, that many of them were reared and crossed with progeny from other wild rats. Selection of the best litters in each generation to propagate the race tended to preserve the descendants of female 1 and her mate, and at the ninth generation they comprised nearly 88 per cent of all individuals in the strain. On tracing the pedigrees of the various mutant types that appeared, it was found that in each case the line of descent went back, unbroken on the maternal side, to the first pair of wild rats. Our present concept of the mechanism of inheritance does not permit of an assumption that this pair of apparently normal gray rats could have carried genes for all the recessive color mutations found. The dominant curly gene would have manifested its presence in the F_1 generation if it had been carried either by female 1 or by her mate. Considering the fact that, aside from the hooded rats, captive grays bred true to type until the eleventh generation, I am of the opinion that these various mutations had their origin in gene changes that occurred after the strain had been in captivity for several years.

Recent work in genetics has shown that gene mutations can be induced by x-rays, radium and other media, but as yet the agencies are unknown that cause such mutations in animals living under natural conditions, or in those maintained in laboratories under fairly uniform conditions of environment and nutrition. The suggestion, advanced years ago, that age changes in the variability of the germplasm may have an important role in the production of such mutations did not receive much support until Little ('33) cited various series of data indicating that there is an age-variability relation not only in the reactions of somatic tissues, but in the germ cells as well. Thus young and old mice exhibit an increased tolerance of tumor transplants as compared with that of young adults (Little, '20; Strong, '22). Variability in the litter size of inbred mice, as shown by coefficients of variation, changes with the age of the mother, being very high among the early litters cast, decreasing to a base level and subsequently rising in litters produced toward the end of the reproductive period.

In full accord with these findings for mice is the series of coefficients for litter size in captive gray rats, as given in table 10 of the present paper. Little ('33) states, "on a priori grounds there is no reason why the germ cells, as an organization, should not also show a durational phase in its various activities." In this connection Bridges ('29) has given data indicating that in Drosophila crossing-over varies with the age of the mother, the greatest variation being shown by very young and by very old females. Haldane and Crew ('25) found that in poultry linkage between two dominant sex-linked factors in males decreased steadily and had practically disappeared by the third year. This finding, they say, "is of interest as pointing to pre-senile changes in the behavior of the dividing nucleus, and as being the clearest case so far recorded in vertebrates of a change with age of the 'germ-plasm' of an individual."

That very young and very old germ cells may function more variably than do those of the middle age period seems further substantiated by the fact that in man some types of defects, which seemingly have an hereditary basis, are found more commonly among children of young mothers, while other types appear more frequently among the late-born. Pearson's ('14) analysis of a large series of data relative to the relation of the order of birth to various defects showed that congenital cataract, albinism, mental defects, tuberculosis and criminality have a particular tendency to occur among the first or second children. On the other hand, Mongolism shows a disproportionate frequency among the late-born, particularly in large families, as many investigators besides Pearson have shown (Langdon-Down, '06; Davenport and Allen, '25; Van der Scheer, '27; Penrose, '32, '33; Murphy, '36, etc.).

In the further development of his thesis Little ('33) also states:

It might well develop that tendency to mutation, which in itself is an indication of variability, would be greater in very young and in very old germ cells. If this were true, domestication, in which very young and very old animals, protected

from the competitive factor of natural selection, are used as parents, would provide a far greater chance for mutations to occur than would natural conditions.

In this hypothesis may lie the explanation for the appearance of various mutations in captive gray rats after they had been under domestication for several years.

In early generations the breeding of gray rats was restricted mainly to the middle and most active period of reproductive life, therefore germ cells in young and old individuals had little chance to function. Females of the first nine generations did not cast their first litters until they were, on the average, over 7 months of age, and reproductive life averaged less than 8 months. In the period when mutations appeared breeding began at an earlier age and was continued much longer than in earlier generations (table 7). Three of the mutant types obtained, black, albinism, and ruby-eyed dilute, first appeared in litters cast by females that were from 14 to 16 months old, and so near the end of reproductive life. The first curly mutant was born when the mother was about 6 months of age. These findings, although forming but a very small series, are in accord with Little's hypothesis. Other investigations along this line may further confirm it. It may be true that "mutations are accidents and accidents will happen" (Sturtevant, '37), but to ascertain the basic cause of such 'accidents' still remains a major problem of genetics.

A general discussion of the effects of captivity on gray Norway rats is deferred until the final report, which will be given when data for the fiftieth generation have been obtained.

SUMMARY

The following summary of life processes in gray Norway rats during fourteen years of captivity covers the findings through the twenty-fifth generation. The data analyzed comprise a total of 8685 litters containing 53,077 individuals.

In both sexes of gray rats rate and extent of body growth increased gradually as the generations advanced (tables 1 to 3, figs. 1 and 2). At the last generation growth acceleration

during the adolescent period was nearly equal to that found in stock albino rats that have been under domestication for a long period of time.

The growth rate in females during the suckling period was more rapid than that in males, but at all subsequent age periods males grew more rapidly than females.

At the twenty-fifth generation adult rats of both sexes were, on the average, about 20 per cent heavier than individuals of the first generation (figs. 3 and 4).

Rats attaining an adult weight much above the average for all individuals of like sex in the same generation group appeared in increasing numbers as the generations advanced (table 4, fig. 5). The weight increase in these individuals is ascribed, tentatively, to genetic factors that activated growth mainly during adult life. Some of these large rats, especially males, showed a tendency to maim or kill smaller individuals of the same sex.

In both sexes of gray rats body-weight variability decreased as the generations advanced. In males of the last generation variability during early life was about one-half that in males of the first generation, and subsequently was still further reduced (table 5, fig. 6). In females of this generation body-weight variability was less than that in females of the first generation at all age periods, but the decrease was significant only for periods during adult life (table 6, fig. 6). This marked decline in variability is attributed mainly to the method of selecting breeding stock and to inbreeding.

There was no sex difference at birth in the body-weight variability of gray rats, but males were more variable than females during early life and less variable during adult life. As the increased variability in adult females was due, probably, to weight fluctuations resulting from pregnancy and lactation, it is concluded that, on the whole, body-weight variability in males was slightly greater than that in females.

At the twenty-fifth generation the average length of the reproductive period was nearly 8 months longer than the average for the first generation (table 7, fig. 8). This extension was caused by the earlier breeding of the rats and the

continuation of their reproductive activities to a more advanced age (fig. 7). Possible explanation for this lengthening of reproductive life are discussed briefly.

Fertility of gray rats, as measured by litter production, increased steadily, reaching its maximum in the nineteenth generation where females produced an average of 10.18 litters as contrasted with an average of 3.50 litters produced by females of the first generation. A slight decline in litter production during later generations resulted from the unfavorable effects on reproduction of changes in the location of the colony (table 8, fig. 9).

Sterility and low fertility of females, ascribable to the effects of captivity, had disappeared by the tenth generation. Subsequently all females reared were fertile, except those in which the reproductive organs became diseased.

In litters of the second to the twenty-sixth generation the range in litter size was from one to fifteen with the mean at 6.1 (table 9, fig. 10). Average litter size in any generation did not differ materially from the mean for the entire series.

The age of the mothers had a marked effect on litter production, litter size and the variation in litter size. The number of litters cast increased until the females were 7 months old and then decreased steadily until the end of reproductive life (table 10). Average litter size reached its maximum (6.55) when mothers were 8 months old and then declined, reaching its lowest point in litters cast at the end of the series (fig. 11). Variation in litter size was slightly below the mean for litters cast by very young females, and significantly lower in litters produced when females were at the height of reproductive activity. When fertility began to wane, variation in litter size increased gradually and reached its maximum in litters cast near the end of reproductive life (table 10).

The sex ratio showed no definite trend as the generations advanced, and for the entire series was 97.3 ± 0.57 (table 11, fig. 12). This ratio is significantly lower than that (105.2 ± 2.00) found in a strain of albinos maintained under the same conditions of environment and nutrition as the gray rats. A

similar disparity in the sex ratios of white and colored races
of man is noted (table 12).

The sex ratio was not influenced by litter size (table 13).

Data given suggest, but give no definite proof, that there
was a cyclic change in the sex ratio of the young as the age
of the mother advanced (table 14, fig. 13).

Mortality at birth was low in all generations, and only 265
stillbirths were recorded for the entire strain. Mortality was
low, also, during the first year of life, averaging 4.2 per cent
for males and 3.4 per cent for females: 79.9 per cent males
and 76.6 per cent females lived to the age of 20 months. The
mortality rate among older rats decreased as the generations
advanced, indicating that under conditions of captivity the
life span in both sexes was lengthened (table 15). The chief
causes of death were pneumonia, which was more prevalent
among males, and tumors which occurred mainly in females.

At the twenty-fifth generation gray rats had lost the high
nervous tension and fear of man displayed by individuals in
early generations. Females then took excellent care of their
young and would serve as foster mothers if certain precau-
tions were taken. Two of the outstanding traits in rats of
early generations still persisted: adults continued to show
antipathy to strangers placed in their cage, and exceptionally
large rats, particularly males, were yet prone to attack small
individuals of the same sex. Gray rats of later generations
could be rendered as tame as are stock albinos if they were
handled frequently during early life. It was then possible
to use these rats for any kind of experimental work.

Several mutations affecting the color or the structure of
the hair appeared in this strain of gray rats. Gray hooded
rats were developed by selective breeding among the descend-
ants of one pair of wild rats in the foundation stock that
carried the hooded gene (figs. 14 to 20). Black, albino and
ruby-eyed dilute mutants appeared in the twelfth to the four-
teenth generations. Curly, a dominant mutation previously
unknown in rats, emerged in the seventeenth generation (figs.
21 to 26). Another new mutation, cinnamon, was found in
the twenty-second generation.

Reasons are given for attributing all these mutations, except hooded, to gene changes that occurred after the rats had been in captivity for some years, and a tentative explanation for their appearance is offered.

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PLATES

EXPLANATION OF FIGURES

14   Ventral view of a rat showing location and size of the white area that indicated the presence of the hooded gene in female 2 of the foundation stock.

15 to 19   Stages in the development of the hooded pattern.

20   Coat pattern at final stage.

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PLATE 1

LIFE PROCESSES IN CAPTIVE GRAY RATS
HELEN DEAN KING

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LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS). II[1]

BY HELEN DEAN KING AND W. E. CASTLE

WISTAR INSTITUTE AND UNIVERSITY OF CALIFORNIA

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King[2] has recently described the occurrence and inheritance of waltzing in an inbred race of rats reared at the Wistar Institute. Concerning the mode of inheritance this statement was made: "Available evidence seems to indicate that waltzing is due to a single gene, but that the extent to which this gene expresses itself depends upon modifying factors which favor or inhibit its action." Selection of the most pronounced waltzers through a series of generations increased the incidence of waltzing. In the F_6 generation the percentage of waltzers produced by waltzing parents had risen to 93 per cent.

The waltzing mutation made its first appearance in albino individuals and though it has since been introduced into colored individuals, it consistently occurs in a higher percentage of the albino than of the colored individuals in litters containing both sorts. This indicates that it is linked with albinism and that its gene is borne in the same chromosome as the albino gene. The evidence for this conclusion is as follows:

Crosses were made between albino waltzers and colored non-waltzers of the varieties known as hairless, kinky and blue. All F_1 individuals were, as expected, colored non-waltzers. The F_2 population obtained from each

cross is classified in table 1 as regards the occurrence of albinism.   No indication was found of linkage between waltzing and the other mutant characters involved in these crosses, hairless, kinky and blue.

TABLE 1

F_2 POPULATIONS IN WHICH IS SHOWN THE COMPARATIVE INCIDENCE OF WALTZING AMONG ALBINO AND NON-ALBINO INDIVIDUALS, RESPECTIVELY

	ALBINO NON-WALTZERS	ALBINO WALTZERS	PER CENT WALTZERS	COLORED NON-WALTZERS	COLORED WALTZERS	PER CENT WALTZERS
Cross 1. Albino waltzer ✕ hairless	82	7	7.8	202	3	1.5
Cross 2. Albino waltzer ✕ kinky	36	8	19.0	154	10	6.1
Cross 3. Albino waltzer ✕ blue	88	13	14.7	316	22	6.5

It will be observed that the incidence of waltzing was in all three of the F_2 populations higher among albinos than among their colored sibs.

The same relation was, with a single exception, found to hold in certain back-cross populations produced by mating F_1 colored non-waltzers with albino waltzers, as shown in table 2.

TABLE 2

COMPARATIVE INCIDENCE OF WALTZING IN ALBINO AND COLORED INDIVIDUALS IN BACK-CROSS POPULATIONS

	ALBINO NON-WALTZERS	ALBINO WALTZERS	PER CENT WALTZERS	COLORED NON-WALTZERS	COLORED WALTZERS	PER CENT WALTZERS
Cross 4A. Albino waltzer ✕ Curly	104	21	16.8	117	21	15.2
Cross 4B. Albino waltzer ✕ Curly	24	18	42.8	35	9	20.4
Cross 5A. Albino waltzer ✕ Black	44	16	26.6	64	16	20.0
Cross 5B. Albino waltzer ✕ Black	58	10	14.7	63	12	16.0
Cross 7. Albino waltzer ✕ Curly₂	52	38	42.2	47	24	33.8
Totals	282	103	26.7	326	82	20.1

In the Cross 5B population alone was the percentage of waltzers as high among the colored as among the albino individuals.   For all the populations of table 2 combined, 26.7 per cent of the albinos were waltzers, but of the colored individuals only 20.1 per cent.

One further point of interest should be noted in connection with Cross 3, table 1.   The blue parent to this cross was heterozygous for yellow, a recessive gene borne in the same chromosome as the albino gene.   If waltzing is linked with albinism, it should show linkage also with yellow.

The F_2 population derived from Cross 3 may be separated into three groups as shown in table 3.

<div align="center">TABLE 3</div>

<div align="center">A FURTHER CLASSIFICATION OF THE F_2 POPULATION OF 426 INDIVIDUALS DERIVED FROM CROSS 3, TABLE 1</div>

	NON-WALTZERS	WALTZERS	PER CENT WALTZERS
Gray, black or blue (i.e., non-yellow colored)	259	16	5.8
Yellow or cream (dilute yellow)	57	6	9.5
Albino	75	13	14.7
Totals	391	35	8.2

As expected, the percentage of waltzers in the yellow group is higher than that in the non-yellow group, suggesting linkage of waltzing with yellow as well as with albinism.

The data presented in tables 1–3 had been obtained prior to March 17, 1936. Subsequent experiments support the tentative conclusion reached at that time, that linkage exists between waltzing and albinism. See table 4.

<div align="center">TABLE 4</div>

<div align="center">FURTHER DATA ON THE COMPARATIVE INCIDENCE OF WALTZING AMONG COLORED AND ALBINO SIBS IN BACK-CROSS POPULATIONS</div>

	ALBINO NON-WALTZERS	ALBINO WALTZERS	PER CENT WALTZERS	COLORED NON-WALTZERS	COLORED WALTZERS	PER CENT WALTZERS
Cross 7A. Albino waltzer × Curly$_2$	171	116	40.4	176	92	34.3
Cross 5C. F_1 (Albino waltzer × black) × black waltzer	30	9	23.1	167	43	20.5
Cross 5D. Albino waltzer × black	146	38	20.6	159	38	19.3
Cross 2A. Albino waltzer × kinky	56	21	27.3	68	16	19.0
Totals	403	184	31.3	570	189	24.9

In each of the four back-cross populations of table 4, the percentage of waltzers is higher among albinos than among their colored sibs, the totals being 31.3 per cent among the albinos to 24.9 per cent among the colored.

Further experiments to test for linkage between waltzing and yellow are as yet incomplete and inconclusive. The total back-cross population obtained by summarizing the various experiments described in tables 2 and 4 consists of 2139 individuals, which fall into the following classes:

	ALBINO NON-WALTZERS	ALBINO WALTZERS	COLORED NON-WALTZERS	COLORED WALTZERS
Observed numbers	685	287	896	271
Corrected for overlapping	463	550	606	519

When we attempt to calculate the crossover percentage between albinism and waltzing, we are confronted with this difficulty, that the observed number of waltzers (558) is less than the observed number of non-waltzers (1581), with which it should be theoretically equal. This, as already explained, we may assume to be due to the occurrence of normal overlaps among the non-waltzers, i.e., of individuals genetically waltzers but actually not manifesting the waltzing behavior. Nevertheless, we observe that the non-crossover class, colored non-waltzers, is larger than the crossover class, albino non-waltzers, and also that the other non-crossover class, albino waltzers, is larger than the other crossover class, colored waltzers, these being the relations which we should expect if linkage exits between albinism and waltzing.

If we assume that overlapping to normality occurs equally among the albino and the colored waltzers, we may use the observed frequencies in calculating a crossover percentage.

The observed number of crossovers will accordingly be the sum of the albino non-waltzers (685) plus the colored waltzers (271), or 956, which is 44.7 $\pm$ 0.7 of the total population, 2139. This is a very loose indicated linkage but one of undoubted significance in view of the small probable error and the consistency of the occurrence of a larger percentage of waltzers among albino than among colored individuals in eight out of nine of the back-cross populations.

This calculation of the crossover percentage may be checked by actually deducting from the classes of non-waltzers, and adding to the classes of waltzers, enough individuals (considered as normal overlaps) to make the total in each of the two groups equal to the half population, 1019. Such a "correction for overlapping" has been made in the foregoing summary of the backcross populations. If we base a calculation of the crossover percentage on these corrected numbers, we get 463 + 519 = 982 as the total of the crossover classes, which is 45.8 $\pm$ 0.7 per cent of the entire population, again a statistically significant indication of linkage.

It has been known for some time that the albino chromosome of the rat contains genetic loci for two other mutant characters, red-eyed yellow (r) and pink-eyed yellow (p).[3] We are now warranted in assuming the existence in this same chromosome of a fourth gene which in its mutant form may produce waltzing. The order of the four genes and their approximate map-distances are as follows:

C	R	P	W
0	0.3	20	45

The experiments described in this paper have been made by King at the

Wistar Institute in consultation with Castle, who has formulated this report.

[1] See also *Proc. Nat. Acad. Sci.*, **21**, 390–399 (1935).

[2] King, H. D., *Jour. Mammology*, **17**, 157–163 (1936).

[3] Castle, W. E., *Genetics and Eugenics*, 4th Ed., 234 (1930).

King, Helen Dean

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Sex ratios   Litter size
Age of mother, effect on sex ratio in young
Rat: albino, Norway, and extracted strains

LITTER PRODUCTION AND THE SEX RATIO IN VARIOUS STRAINS OF RATS

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

TWO CHARTS

Fertility has been ably defined by Pearl and Surface ('09) as, "the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring." In accordance with this definition, norms for the fertility in any stock should be derived from the total number of offspring produced by a considerable number of breeding animals, not from random sampling. The latter method, which is too frequently employed, is open to a very serious error. Since only relatively young animals, as a rule, are used for breeding, random sampling of reproduction usually shows the fertility of early life only, and fails to indicate the marked decrease in the litter frequency and in the number of offspring that comes after the animals have passed the zenith of their reproductive activity.

Extensive series of breeding experiments with various strains of rats have furnished data for litter production that cover the entire reproductive life of a large number of individuals. These data, which are given in the present paper, are for the following strains: 1) stock Albinos; 2) Norway rats, born and reared in captivity; 3) piebalds, a black-hooded variety; 4) extracted Albinos; 5) extracted Norways. As all of these strains of rats were reared in The Wistar Institute animal colony under similar conditions of environment and of nutrition, differences in their fertility and in their sex

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ratios must be attributed mainly to qualities inherent in the strains.

A definite routine was always followed in obtaining litter data for the various strains. Cages containing breeding animals were inspected at least once a week. If any females were found to be pregnant the cage was marked and examined daily, if possible, until the litter was born. As the sexes can readily be distinguished at birth (Jackson, '12), sex data were noted on record cards at the time that the litter was discovered. By this method the birth of a litter and the sex of the young were recorded, in the great majority of cases, within a few hours after parturition. An early record of the litters is necessary if the data are to have much statistical value, since the delay of a few days in examining a litter may mean the failure to record a considerable number of the young. Sometimes a mother destroys all or part of a litter shortly after its birth, or she may neglect the young entirely, when they soon die and are either eaten by adult rats or lost in the debris of the cage. If several adults occupy the cage there is the further danger that they will all crowd into the nest and smother some members of the litter, which then meet the fate of neglected young. Because of such possibilities errors of omission must inevitably occur in the records of a long-continued series of breeding experiments with the rat, but they can be reduced to a minimum by promptness in recording the litter data.

Data for stillborn young have been included in all litter records. The exclusion of such individuals would not only lessen the average size of the litters, but also change the sex ratio, since it has been shown that stillborn young comprise about 2 per cent of the total number of offspring in albino rats and that among them the males greatly outnumber the females (129 males to 100 females: King, '21).

The length of the reproductive period in the rat varies considerably in different individuals and in different strains, and it is also affected by nutritive conditions (Slonaker and Card, '23 c). As a rule, this period covers from twelve to

fifteen months of the life of the female; no data have been obtained for the male as yet. The extreme limits of reproductive activity that have been noted show the birth of an albino litter when the mother was but fifty-six days of age and the production of a litter by a pigmented female when she was over thirty-three months old. The data given in the present paper include no litters cast by females that died before the approximate end of reproductive life. If, however, a female lived the required length of time and appeared to be in good physical condition, her offspring have been included even though she cast only one litter.

Sex ratios are given in the various tables only when the number of individuals in any group exceeded twenty-five. The probable error of the ratio has been calculated by Yule's ('22) formula:

$$P. E. = \pm 67.45 \,(1+Z)\sqrt{\frac{Z}{N}},$$

Z being the ratio, i.e., the number of males divided by the number of females, and N the total number of individuals.

Since in the latter part of the reproductive period there is a marked decrease both in the number and in the size of the litters cast, it is difficult to obtain sufficient data for young born during this time to give sex ratios having much statistical value, even when a large number of breeding females are available. As sex ratios in litters cast by old females often deviate widely from 100, the probable error of these ratios, as calculated by Yule's formula, is very large and their use certainly tends to conservatism in considering the significance of the results.

LITTER PRODUCTION AND THE SEX RATIO IN STOCK ALBINOS

The data for this strain were obtained from the records of two large series of animals descended from Albinos taken from the general stock colony maintained at The Wistar Institute.

As stated by Donaldson ('15), albino females usually begin breeding when they are three or four months old, and the

menopause comes when they are from fifteen to eighteen months of age. Data given for this strain comprise litters cast by females that lived to be at least sixteen months old. Many of the females were kept until they were nearly two years old, but only a very few of them cast litters after they were over eighteen months of age.

Data for litters produced by 148 stock albino females during the period of their reproductive activity are given in table 1.

TABLE·1

Data for entire series of litters cast by 148 stock albino females

LITTER SERIES	NO. LITTERS	NO. INDI-VIDUALS	AVERAGE NO. YOUNG PER LITTER	MALES	FEMALES	NO. MALES TO 100 FEMALES
1	148	903	6.1	462	441	104.7 ± 4.70
2	148	992	6.7	498	494	100.8 ± 4.32
3	142	876	6.1	454	422	107.5 ± 4.88
4	128	824	6.4	402	422	95.2 ± 4.45
5	98	547	5.5	279	268	104.0 ± 5.99
6	64	344	6.4	184	160	115.0 ± 8.38
7	38	228	6.0	128	100	128.0 ± 11.37
8	23	137	5.9	72	65	110.7 ± 12.64
9	13	85	6.5	51	34	150.0 ± 22.39
10	5	22	4.4	13	9	
11	3	12	4.0	5	7	
12	3	13	4.3	8	5	
13	2	9	4.5	3	6	
1–13	815	4992	6.1	2559	2433	105.2 ± 2.00

Table 1 brings out clearly a fact that has already been noted by several observers (Crampe, '83; King and Stotsenburg, '15; Slonaker and Card, '23 c), namely, that the second litter tends to be the largest of the series cast by albino females; the third and fourth litters, as a rule, being but little smaller than the second. If an albino female is in good condition she usually casts her fifth litter when she is seven or eight months old, at which time she has reached the height of her reproductive activity and has produced approximately one-half of her total number of offspring (King, '16). As the litter series advances beyond this point, there is a gradual decline in the number of young cast at each

birth. In table 1 the marked decline in litter size comes at the tenth litter where the number of young drops to 4.4— the average that is maintained until the end of the series.

For the entire series of 815 litters recorded for the albino strain the average number of young per litter was 6.1. This average seems very low, since it is generally asserted that litters of albino rats usually contain about seven young. The data given in table 1, as already stated, are a selected lot based solely on the fact that the mothers of the litters lived throughout the normal length of the reproductive period. Where an average of seven or more young has been obtained for a large series of albino litters the data have been taken from the litter production of relatively young females and, therefore, fail to show the lesser fertility of animals approaching the menopause.

Cuénot ('99) gives the normal sex ratio for the albino strain as 105.6 males to 100 females; in the data collected by King and Stotsenburg ('15) the sex ratio was 107.5 males to 100 females; Slonaker and Card ('23 d) have recently given the sex ratio in a series of 944 Albinos as 108.3 males to 100 females. The first of these determinations was based on records for only thirty litters; the second on a random collection of 1089 litters produced in a colony of relatively young animals. The data of Slonaker and Card, although covering a wider span of the reproductive life of the mothers than either of the other series of records, are not complete, as the authors state that "the sexes were not determined in all of our litters." None of these sex ratios, therefore, can properly be taken as a norm for the albino strain.

In table 1 the sex ratios are given for only the first nine litters of the series, since the number of young produced in later litters was too small to give ratios having statistical value. To attempt an analysis of the ratios for the various litter groups would be futile. The ratio for one litter group bears seemingly no relation to the ratio for the group immediately preceding or following, and the differences between successive ratios, when judged by their probable error, are

of little import. The ratios seem, however, to indicate that the number of males tends to increase as the litter series advances up to the tenth litter. Beyond this point the relative number of males decreases sharply, as the data for the last three litters of the series, when combined, give a sex ratio of only 88.8 males to 100 females.

For the total of 4992 albino young recorded in table 1 the sex ratio is 105.2 males to 100 females. This ratio is but slightly less than that given by Cuénot, and may, perhaps, serve as a norm for sex ratio in the albino strain until a larger and more complete series of data is available.

LITTER PRODUCTION AND THE SEX RATIO IN THE NORWAY RAT

The strain of Norways now undergoing the process of domestication in The Wistar Institute animal colony was developed from wild Norway stock trapped in the vicinity of Philadelphia in 1919. The data given cover the litter production of females in the first three generations, born in captivity, that lived to be at least twenty months old.

Three investigators have recorded observations regarding the reproductive activity of the Norway rat. Crampe ('84) makes the general statement that Norway rats develop their reproductive power much more slowly, reach a maximum later and maintain it longer than do Albinos. Lantz ('10) states that in their natural habitat Norway rats are capable of breeding when they are less than three months old, though he does not give the data on which this assertion is based. According to the observations of Miller ('11), sexual maturity is attained by both sexes of Norways by the end of the fourth month when the animals are born in captivity. In my strain of Norways some of the females belonging in the early generations did not cast a litter until they were twelve months old, and the average age at which these Norway females, as a group, began breeding was about eight months; in later generations a number of females have cast litters when from four to five months of age. In captivity the menopause in the Norway strain comes when the females are

about twenty months old, though several females have cast litters when nearly two years old.

Darwin ('75) cites many instances where the fertility of wild animals was greatly lessened in captivity, though the animals did not lose their vigor and their reproductive organs were not diseased. The wild Norway stock from which my strain was derived exhibited in a marked degree this tendency to sterility under conditions of captivity. Of the twenty wild Norway females brought into the colony at the beginning of this investigation, only six were known to breed, although most of the animals were kept in good condition for many

TABLE 2

Data for entire series of litters cast by eighty-eight Norway females, born and reared in captivity

LITTER SERIES	NO. LITTERS	NO. INDI-VIDUALS	AVERAGE NO. YOUNG PER LITTER	MALES	FEMALES	NO. MALES TO 100 FEMALES
1	88	462	5.2	223	239	93.3 ± 5.85
2	84	547	6.5	253	294	86.0 ± 4.96
3	63	410	6.5	190	220	86.3 ± 5.75
4	39	229	5.9	106	123	86.1 ± 7.69
5	20	128	6.4	58	70	82.8 ± 9.91
6	10	62	6.2	21	41	51.2 ± 9.26
7	5	24	4.8	9	15	60.0 ± 17.17
1–7	309	1862	6.0	860	1002	85.8 ± 2.68

months. To what extent changed conditions of environment and of nutrition affected the length of the reproductive period and the fertility in the early generations of Norways born in captivity cannot be determined, since practically nothing is known of the reproductive activity of these animals in their natural state. My Norways have rapidly adapted themselves to change in habitat, and they seem to live in captivity quite as well as do the domesticated Albinos. Their present environment seems favorable to growth and to longevity, and apparently has had no marked effects on normal life processes.

Data for 309 litters cast by 88 Norway females are shown in table 2.

In the Norway, as in the albino strain, the first litter cast is relatively small and the next two litters tend to be the largest of the series cast. In later litters the number of young gradually decreases, dropping to an average of 4.8 in the final litter of the series (table 2). The 309 litters cast by Norway females contained an average of 6.0 young: the data for the albino strain gives an average of 6.1 young per litter (table 1). The average size of the litter is, therefore, practically the same in the two forms; but as litter production in the albino strain is greater than that in the Norway strain it appears that domestication has increased the fertility of the rat, as it has of many other animals (Darwin, '75).

The greatest number of litters produced by any Norway female was seven. Four of the females, as table 2 shows, cast but one litter each, although all lived to an advanced age. Since Norway females in captivity often devour their young, as Miller ('11) has noted, there is the possibility that these four females cast other litters that were not recorded. This supposition seems improbable, however, as the nests were examined frequently to guard against such an occurrence.

The papers of Lantz ('10) and of Miller ('11) give practically all of the information previously recorded regarding litter size in the Norway strain. Lantz cites instances in which twenty-two and twenty-three young were found in a single nest in England, but as female rats frequently pool their young, these records do not necessarily indicate that the young were the progeny of a single female. An examination of a large number of pregnant females by The Plague Commission in India showed that the average number of embryos was 8.1, the highest number found being fourteen. Lantz further mentions two cases where seventeen and nineteen embryos were found in pregnant Norways, and infers that in this latitude the average litter is not under ten. Since in the rat, as the observations of Huber ('15) and of Long and Evans ('22) have shown, many fetuses die at various stages of gestation and are absorbed in situ, the number of embryos found in gravid females is no safe criterion by which to judge the number of living young that will be born.

In eight litters obtained from wild Norway parents Miller ('11) found that the number of young varied from seven to twelve, with an average of 10.5. The mothers of these litters were females of unknown age. Miller states that the Norways breed during all months of the year—an observation which my investigations confirm. The period of greatest litter production, however, seems to be in the spring and summer, few litters being cast during the autumn months.

The sex ratios for litters in the Norway strain, as given in table 2, differ greatly from those obtained for the stock Albinos (table 1). In all litters of the Norway series the sex ratios are very low, ranging from 51.2 to 93.3 males to 100 females. As the probable errors of these ratios are large, the difference between any two succeeding ratios are not great enough to be significant. Between the highest ratio, that for the first litter group, and the lowest ratio, found in the sixth litter group, the difference of 42.1 ± 10.95 is sufficiently great, however, to indicate that old females tend to produce a relatively greater number of female than of male young.

For the total of 1862 Norway young recorded in table 2 the sex ratio is but 85.8 males to 100 females. This ratio is in accord with the only other sex ratio for the Norway strain that has as yet been recorded, namely, the ratio of 82.1 males to 100 females found by Miller in five litters comprising fifty-one young.

LITTER PRODUCTION AND THE SEX RATIO IN EXTRACTED STRAINS OF RATS

All of the extracted strains of rats used in this study were derived from the F_2 generation of a cross between wild Norway males and stock albino females. In each strain several successive generations were reared and the growth and fertility of a considerable number of individuals determined. Animals in these strains did not begin breeding at quite as early an age as did the stock Albinos, but the majority of females that were fertile cast their first litter when they

were from four to five months old; the menopause appeared twelve to fifteen months later regardless of the coat color of the individuals. Data given are for litters cast by females that lived to be at least eighteen months old.

1. Extracted Albinos

Although extracted Albinos were easily handled and possessed none of the vicious traits of the Norway rats, they nevertheless showed traces of their wild ancestry in that they

TABLE 3

Data for entire series of litters cast by fifty-seven extracted albino females

LITTER SERIES	NO. LITTERS	NO. INDI- VIDUALS	AVERAGE NO. YOUNG PER LITTER	MALES	FEMALES	NO. MALES TO 100 FEMALES
1	57	320	5.6	167	153	109.1 ± 8.22
2	57	349	6.1	191	158	120.9 ± 8.76
3	56	348	6.2	166	182	91.7 ± 6.63
4	46	277	6.0	142	135	105.1 ± 8.52
5	28	153	5.3	79	74	106.7 ± 11.72
6	11	71	6.4	33	38	86.8 ± 13.92
7	6	41	6.8	21	20	105.0 ± 22.12
8	2	15	7.5	11	4	
9	2	11	5.5	4	7	
10	1	5	5.0	3	2	
11	1	8	8.0	4	4	
1–11	267	1598	5.9	821	777	105.6 ± 3.56

were more active than stock Albinos. They also exhibited a tendency to gnaw their way through any part of the cage that was unprotected with wire—a trait rarely shown by stock animals.

Litter data for this strain are given in table 3.

The extracted albino strain is the only one of the five under investigation in which the second litter was not the largest of the series cast. Litters produced when the females were from eight to ten months old tended to be larger than those cast by younger individuals; later litters showed a gradual decrease in the number of young (table 3). The entire series of 267 litters contained an average of 5.9 young, which is

slightly lower than the average for the stock albino strain
(6.1—table 1).

Sex ratios for the litters cast in this strain vary consider-
ably and show no pronounced change with the advance of
the series. Differences between successive ratios cannot be
deemed significant when judged by their probable errors.
For the entire series of litters the sex ratio is 105.6 males
to 100 females. This ratio differs little from that for the
stock Albinos (table 1).

TABLE 4

Data for entire series of litters cast by fifty extracted Norway females

LITTER SERIES	NO. LITTERS	NO. INDIVIDUALS	AVERAGE NO. YOUNG PER LITTER	MALES	FEMALES	NO. MALES TO 100 FEMALES
1	50	317	6.3	153	164	93.2 ± 6.56
2	50	386	7.7	204	182	112.1 ± 7.69
3	50	361	7.2	192	169	113.6 ± 8.07
4	40	226	5.6	119	107	111.2 ± 9.98
5	22	168	7.6	88	80	110.0 ± 11.44
6	15	100	6.6	53	47	112.7 ± 15.22
7	11	67	6.1	29	38	76.3 ± 12.68
8	8	45	5.6	24	21	114.3 ± 23.02
9	6	28	4.6	15	13	115.4 ± 29.49
10	2	14	7.0	8	6	
11	2	11	5.5	6	5	
12	2	11	5.5	6	5	
13	2	6	3.0	2	4	
1–13	260	1740	6.7	899	841	106.9 ± 3.46

2. *Extracted Norways*

Aside from their coat color, extracted Norways showed
none of the pronounced traits of their wild ancestry. They
were as tame and as easily handled as are Albinos, and very
few of them exhibited a tendency to gnaw the cage or to de-
stroy their young. Breeding began in this strain somewhat
later than in the other extracted strains, as most of the fe-
males did not cast a litter until they were about five months
old.

Data for litter production in extracted Norways are given
in table 4.

In the series of litters cast by extracted Norways the second litter was the largest, containing an average of 7.7 young. From this point litter size decreased gradually as the females approached the end of the reproductive period. The females in this strain showed a high degree of fertility, as is indicated by the number of litters cast and by the fact that the average number of young in the entire series of litters was 6.7.

The sex ratios for the litter series of extracted Norways show a range of variation of from 76 to 115 males to 100 females, the highest ratio being that for the ninth litter group. In the total of 260 litters the sex ratio was 106.9 males to 100 females. That this ratio is relatively high is due, perhaps, to the fact that in the first two or three generations some of the individuals used for breeding were hybrids, as was shown by the appearance of albino young in the litters cast. In hybrids between Norways and Albinos the sex ratio tends to be very high (King, '11). In later generations hybrids were eliminated and all individuals bred true to type. It did not seem advisable to discard from these records litters in which albino young were cast, since such an elimination would have reduced the total number of litters, and the sex ratio for the strain, as given in table 4, would have been lessened by at most one point.

3. Piebalds

Of all strains of rats at any time under investigation the piebalds, a black-hooded variety, have been the most fertile, they have lived the longest and have shown the greatest resistance to the rat scourge, pneumonia.

Litter data for the piebald strain are given in table 5.

The effect of the age of the mother on litter size is brought out with great clearness in the long litter series shown in table 5. The second litter, with an average of 8.2 young, is the largest of the series. Litter size decreases but slowly up to the eighth litter group, where it drops to 5.8; later litters are still smaller, the final one containing but two young. Even in this strain of piebalds, which showed unusual fer-

tility, the average number of young in the 412 litters cast was
only 6.8. Had the fertility of the strain been determined by
the method of random sampling, the error in the finding would
have been very great.

As in the other strains under investigation, the sex ratios
for the litter groups in the piebalds show a wide range of
variation, and the size of the probable errors makes the dif-
ferences between the ratios of successive groups practically
valueless. The ratios are relatively low for the early litters,

TABLE 5

Data for entire series of litters cast by fifty-one piebald (black-hooded) females

LITTER SERIES	NO. LITTERS	NO. INDI- VIDUALS	AVERAGE NO. YOUNG PER LITTER	MALES	FEMALES	NO. MALES TO 100 FEMALES
1	51	381	7.4	198	183	108.2 ± 7.44
2	51	420	8.2	197	223	88.3 ± 5.80
3	51	364	7.1	195	169	115.4 ± 8.17
4	51	395	7.7	193	202	95.5 ± 5.45
5	46	343	7.4	178	165	116.9 ± 8.52
6	39	268	6.8	·140	128	109.3 ± 9.00
7	31	228	7.3	121	107	113.0 ± 10.11
8	27	158	5.8	88	70	125.7 ± 13.56
9	22	112	5.1	70	42	166.6 ± 21.75
10	19	83	4.3	47	36	133.3 ± 19.82
11	11	48	4.3	21	27	77.7 ± 15.22
12	7	24	3.4	9	15	
13	5	19	3.8	7	12	
14	1	2	2.0	0	2	
1–14	412	2845	6.8	1464	1381	106.0 ± 2.68

rising to a maximum of 166.6 males to 100 females for the
ninth litter group. In the next group the ratio drops 33
points, falling still further in the eleventh litter of the series
where there were but 77.7 males to 100 females. For the
entire series of litters, comprising 2845 individuals, the sex
ratio is 106.0 males to 100 females.

None of the final sex ratios for the litters of extracted
strains of rats differ significantly from the ratio for the
stock Albinos. There is therefore no evidence that extracted
strains of rats inherit from their wild ancestry genetic factors
that materially influence the sex ratio in the young at birth.

LITTER PRODUCTION IN VARIOUS STRAINS OF RATS

The total number of litters cast by different females in any strain of rats is extremely variable. Some females will produce a litter every month or two during the entire reproductive period; others will cast but one or two litters, although they are apparently in good health and live to an advanced age. Litter production in the rat seems to depend to a great extent on the physical condition of the animals and on nutrition. Underfed animals, or those not on a well-balanced diet, have but few litters which contain only a small number of young (Slonaker and Card, '23 b). The outstanding factor that is largely responsible for checking fertility in any large colony is the so-called 'pneumonia' which attacks adult animals of any age, but chiefly those over a year old. There is considerable evidence, also, that infection in the uterus and in the tubes lessens the number of litters in many cases (Long and Evans, '22).

As conditions of environment and of nutrition were practically the same for all strains of rats used in this study, and as no strain showed a marked susceptibility to disease, the findings for litter production here given may, perhaps, be considered as fairly representative for the various groups. The number of litters cast by females in the various strains are shown in table 6.

The greatest range in the number of litters cast, as the data in table 6 show, is found in the stock Albinos, and these females, as a group, produce an average of 5.5 litters. On the other hand, the most restricted range in litter production occurs in the Norway strain. Four of these females cast only one litter each, and none of them had more than seven litters; the average for the group being only 3.5. This finding for the Norways is due to the fact that these females began breeding at a much later period than did the females in other strains. Litter production in the extracted Albinos and in the extracted Norways shows a range of variation and an average somewhat less than that for the stock Albinos, but considerably greater than that for the pure Norway strain. The piebalds

TABLE 6

Number of litters cast by females in various strains of rats

NUMBER LITTERS CAST	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL NUMBER LITTERS	AVERAGE NUMBER LITTERS
Stock Albinos......		6	14	30	34	26	15	10	8	2			1	2		815	5.5
Norways	4	21	24	19	10	5	5								309	3.5
Extracted Albinos		1	10	18	17	5	4		1		1				267	4.7
Extracted Norways			10	18	7	4	3	2	4				2		260	5.2
Piebalds..........				5	7	8	4	5	3	8	4	2	4	1	412	8.1
	4	28	58	90	75	48	31	17	16	10	5	3	8	1	2063	5.2

showed a great superiority over the other strains as regards litter production; none of the females cast less than five litters, and the average for the group was 8.1.

From the data for the total number of litters produced in all strains the graph in chart 1 has been constructed.

The graph in chart 1 rises in a straight line to its nodal point at 4 and then gradually declines, ending at the four-
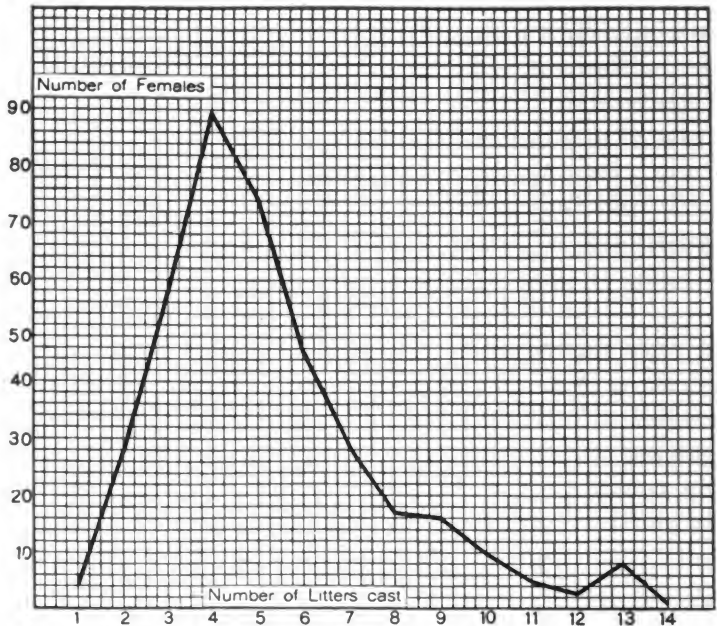
Chart 1 Showing the graph for litter frequency in the rat. Constructed from the summary of all data as given in table 6.

teenth litter cast. The finding shown in this graph confirms the observation of Crampe ('84) that rats, in general, produce an average of only four or five litters during the course of their reproductive life.

LITTER SIZE IN VARIOUS STRAINS OF RATS

Litter size in the rat depends to a considerable extent on conditions that tend to influence fertility in general; the physical condition and age of the mother being the two chief factors.

Any disease that tends to lessen the vitality of the female has a marked effect on the number of offspring produced. For example, 'pneumonia' lessens the number of young in the litters cast and causes an increase in birth mortality (King, '15), while infection in the ovary or tubes first decreases the number of young and subsequently leads to complete sterility (Long and Evans, '22). The effect of the age of the mother on litter size is shown by the data given in tables 1 to 5. The first litter cast by a young female is, as a rule, relatively small, ranging from three to seven young, while the second litter tends to be the largest of the series. The number of young per litter decreases gradually from this time, and after a female has reached the age of one year she rarely produces a litter containing over eight young, the average being four or five. This general statement regarding the number of young cast applies only to a large group of litters, since individual females show great variation in the number of young produced at all ages.

Frequencies of litter size in the various strains of rats are shown in table 7.

In the stock Albinos the litters varied in size from one to twelve, the majority containing from five to seven young. Albino litters of thirteen to sixteen young have frequently been reported, and litters of seventeen have been obtained in other strains of Albinos bred in our colony. The range in litter size for the Albino strain shown in table 7 does not, therefore, extend to the known upper limit of litter size for the strain in general.

In the Norway strain only one litter was cast that contained as many as thirteen young, litters of six being the most frequent. This finding is contrary to the general belief that litters of Norway rats usually contain ten or more young (Lantz, '10; Miller, '11). How truly the data given in table 7 represent the size of the general run of litters cast by Norways breeding in their natural habitat cannot be determined, since similar data for wild Norways, available for comparison, have been obtained by the method of random sampling

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TABLE 7

Frequencies of litter size in various strains of rats

SIZE OF LITTER	1	2	3	4	5	6	7	8	9	10.	11	12	13	14	15	16	AVERAGE SIZE OF LITTER
Stock Albinos......	7	33	58	116	125	126	121	107	56	37	25	4					6.1
Norways..........		11	29	36	46	65	53	32	20	9	5	2	1				6.0
Extracted Albinos		13	26	31	47	50	32	28	27	8	2		1	1	1		5.9
Extracted Norways	1	4	19	20	33	44	39	47	27	19	3	2	2				6.7
Piebalds..........	6	21	32	35	31	57	40	72	49	29	17	13	8	1		1	6.8
	14	82	164	238	282	342	285	286	179	102	52	21	12	2	1	1	6.4

and do not take into account the probability that two or more females may cast their litters in the same nest.

In all extracted strains the range in litter size was greater than in the Albino and in the Norway strains and the litters tended to be larger, eight being the most frequent number of young in the extracted Norways and in the piebalds.

The average size of all litters cast in each strain is shown in the last column of table 7. The range is from 5.9 to 6.8, with 6.4 as the final average for the total of 2063 litters containing 13,037 individuals. From these data it appears that the entire series of litters cast by a large group of female rats, regardless of strain, averages a little over six young per litter. Litters larger than twelve are exceptional, and very small litters, as records show, are found usually when the mother is at one extreme or the other of the reproductive period or is in poor physical condition.

According to the data given in table 7, graphs plotted to show the frequencies of litter size in the stock Albinos, in the Norways, and in the extracted Albinos would all have the same general form. The nodal point would be at six and the decline in the graph would be more gradual than its rise. Graphs for the extracted Norways and for the piebalds would have a slightly different form: the nodal point would be at eight and the rise in the graph would be more gradual than its decline. From the summary of the data for all strains the graph in chart 2 was constructed.

In chart 2 the graph rises abruptly to its nodal point at six, declines gradually to twelve, and ends at sixteen. The slight rise in the graph at eight is due to the relatively large size of the litters in the extracted Norway and in the piebald strains.

In a previous paper (King, '16) a graph was given for frequencies of litter size in the rat in which there were two nodal points, one at six and the other at eight. The suggestion was made that possibly one nodal point corresponded to the degree of fertility that is normal for the Norway rat and the other to the degree of fertility that characterizes the

albino rat, since at the time this paper was written there were no data available regarding litter size in the Norway rat. This suggestion now seems untenable, as data given in table 7 of the present paper show that apparently there is no appre-

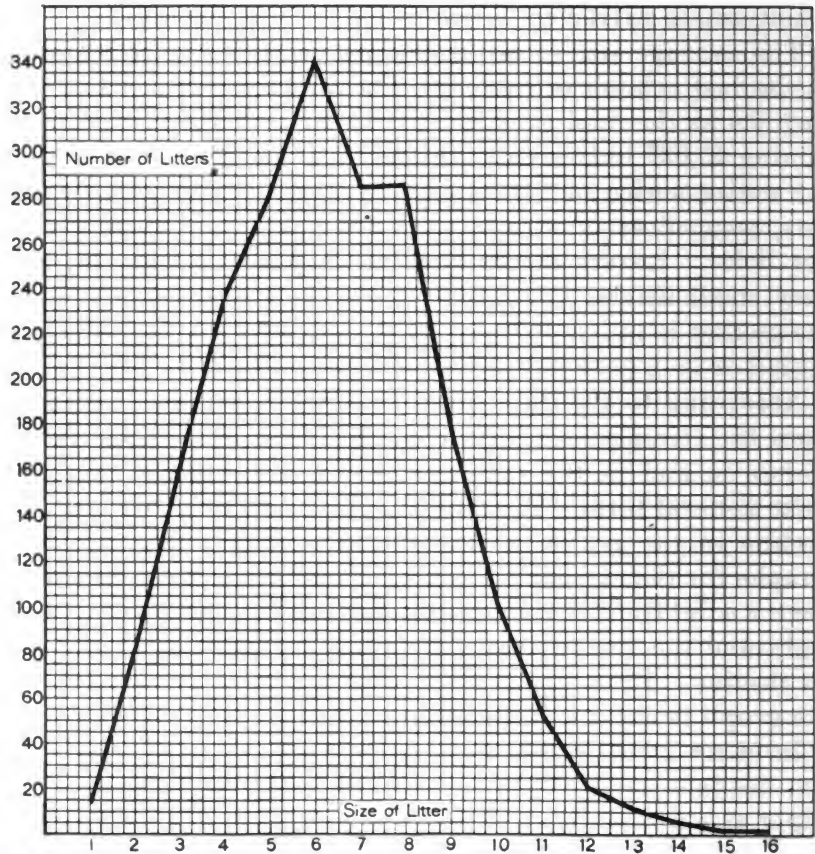Chart 2 Showing the graph for frequency of litter size in the rat. Constructed from the summary of all data as given in table 7.

ciable difference between these two strains as regards the average size of the litters cast. The binodal form of the previous graph, therefore, was doubtless due to the fact that the graph was constructed from comparatively few data taken from several different strains of rats.

The change in litter size that comes with the advancing age of the female rat has been noted by other observers (Crampe, '83; Slonaker and Card, '23 c), and a similar phenomenon has been found to occur in many other animals also. Minot ('91) and Wright ('22) have observed that litter size in guinea-pigs increases during the first part of the reproductive period and then declines. A marked effect of the age of the mother on the number of offspring produced has been found for rabbits (Hammond, '14), for dogs (Marshall, '10), for sheep and fowl (Pearl, '13, '17), and for pigs (Hammond, 14; Carmichael and Rice, '20). Hammond ('14) attributes the low fertility of young sows to the fact that a smaller number of ova are liberated at each ovulation by young females as compared with the number shed by older females. Undoubtedly changes in the uterus due to advancing age affect the implantation of the ova and so account for the small size of litters cast by old females, even though as many ova are shed at this period as at an earlier age.

THE SEX RATIO IN VARIOUS STRAINS OF RATS

As the sex ratios for the various litter series, as given in tables 1 to 5, were calculated from relatively small numbers of data they show such a wide range of variation, and have probable errors of such magnitude, that no definite conclusions can be drawn from them. Whether the proportion of the sexes in the young at birth is the same for all strains of rats or whether each strain has a characteristic sex ratio of its own seems a question of sufficient importance to justify a recombination of the data that may throw some light on this point. It is of interest, also, to determine whether the sex ratios for the various litters of a given strain tend to be alike or whether the ratio changes with the advance in the litter series and therefore with the age of the mother. With these questions in mind, data for each two successive litters were combined for each strain of rats and the sex ratios calculated when the number of individuals in any group exceeded twenty-five. These ratios, with their probable errors, are given in table 8.

Fluctuations due to small number of individual data have been largely eliminated in the series of sex ratios shown in table 8, therefore it is possible to determine more exactly the general trend of the ratios in any given strain.

In the stock Albinos the sex ratio is below the norm in the first two litter groups; it rises gradually to a maximum of 148.8 males to 100 females which is reached in the fifth group comprising the ninth and tenth litters cast. From the maximum there is a drop of nearly 60 points in the sixth and final group. This drop is not significant, however, if judged by the size of the probable error.

The sex ratios for the piebald strain, as given in table 8, show exactly the same general trend as do those for the Albinos, and the agreement between corresponding ratios in the two strains is very close—too much so to be attributed merely to chance.

No very definite conclusions can be drawn from the series of ratios for the extracted albino strain, since ratios could not be calculated for the later litters cast. The maximum ratio of 133.3 males to 100 females, found in the fourth litter group, is in line with the advance in the ratio for the corresponding litter group in the albino and in the piebald strains.

The ratios for the extracted Norways show, on the whole, the same general trend as do the ratios for the Albinos and piebalds. In the fourth-litter group the ratio of 89.8 males to 100 females is at variance with the corresponding ratio in the other strains, but it cannot be deemed important, particularly as the ratio for the following group is very high. In this strain also the sex ratio for the final litter group is low.

Sex ratios calculated from the total number of individuals in each strain vary from 105.2 to 106.9 males to 100 females. This variation is not significant. For the total of 11,175 individuals comprised in all four strains the sex ratio is 105.7 males to 100 females. This ratio is in accord with that for human offspring at birth.

TABLE 8

Sex ratios in various strains of rats, calculated from data in tables 1 to 5 combined by litter groups

LITTER GROUPS	STOCK ALBINOS		PIEBALDS		EXTRACTED ALBINOS		EXTRACTED NORWAYS		ALL STRAINS EXCEPT NORWAYS		NORWAYS	
	Number individuals	Number 100 males to females	Number individuals	Number 100 males to females	Number individuals	Number 100 males to females	Number individuals	Number 100 males to females	Number individuals	Number 100 males to females	Number individuals	Number 100 males to females
1- 2	1895	102.6± 3.17	801	97.2± 4.62	699	115.1± 5.87	703	103.1± 5.24	4008	103.6± 2.19	1009	89.3±3.78
3- 4	1700	101.4± 3.08	759	104.6± 5.12	625	97.1± 5.23	587	112.6± 6.08	3671	103.0± 2.29	939	84.3±4.57
5- 6	891	108.2± 4.93	611	108.5± 5.92	224	100.0± 9.01	208	111.0± 9.15	1994	107.7± 3.24	214[1]	69.8±6.54
7- 8	365	121.2± 8.59	386	118.0± 8.13	56	133.3±24.23	112	89.8±11.45	919	116.2± 5.16		
9-10	107	148.8±19.80	195	150.0±14.78	16		42	121.0±25.29	360	141.6±10.21		
11-14	34	88.9±20.57	93	66.0± 9.42	8		28	100.0±24.95	163	75.0± 8.00		
1-14	4992	105.2± 2.00	2845	106.0± 2.68	1598	105.6± 3.56	1740	106.9± 3.46	11.175	105.7± 1.35	1862	85.8±2.68

[1] Data for seventh litter included.

The accord in the sex ratios for corresponding litter groups in these four strains of rats is as close as could reasonably be expected, considering the widely different numbers of individuals involved. The trend of the ratios is the same in the Albinos and in the piebalds, the two largest series, and that for the two other strains is in the same general direction. These facts warrant the conclusion, I think, that in these strains of rats the sex ratio shows a definite trend with the advance in the litter series: it is below the norm in early litters cast, rises slowly to a maximum, and drops abruptly to a very low point in litters cast at the end of the series. The validity of this conclusion is challenged by the fact that the differences between the sex ratios for successive litter groups are not significant when judged by the size of their probable errors. It would seem, however, that the accumulatuve evidence for all four strains would be sufficient to outweigh the significance of probable errors that admittedly are of little value when numbers of individuals are not large and the ratios vary greatly from equality.

It is of interest in this connection to note that in a series of some 900 albino rats, used by Slonaker and Card (23 d) as controls for their recent investigations on the effects of a restricted diet, the sex ratio rises to a maximum of 163.6 males to 100 females in the sixth litter cast and drops to a low point in later litters. The general trend of the ratios is therefore in accord with those given above.

The sex ratios for the Norway strain are so strikingly different from those for the other strains that they must be considered apart. As shown in table 8, all of these ratios are very low, and their trend is uniformly downward as the litter series advances. There is a difference of 20 points between the maximum ratio, that for the first litter group and the final ratio, but this difference is not significant when judged by the size of its probable error. For the total of 1862 individuals in the Norway strain the sex ratio, 85.8 males to 100 females, is significantly lower than the corresponding ratio for any of the other strains. It is thus evident that Norway

females that have not become domesticated tend to cast relatively fewer male young than do females in the other strains.

It may be maintained that the sex ratio found in this series of Norways is due to the effects of captivity on the breeding animals and that it is not the ratio normal for these animals in their wild habitat. On this supposition the most obvious explanation for the low sex ratio is to assume that there has been selective mortality in fetal life. In the rat, as in many other mammals, male embryos seem to possess less constitutional vigor and less vitality than do female embryos (King, '21), and it might be that restricted activity and changed conditions of nutrition, incident to captivity, so affected the females that proportionately more of the male than of the female embryos died during fetal life, thus changing the sex ratio in the young at birth. This explanation seems untenable, since the sex ratio for individuals cast in later generations of these Norways is changing progressively toward the ratio that is normal for the albino strain. It would appear, from the evidence at hand, that a low sex ratio is normal for the Norway strain, and that the high ratio in the Albinos is due, in part at least, to the effects of domestication. As Norway rats in their natural habitat are polygamous, an excess of female offspring would be of decided advantage in the propagation of the race. This may account for the ever-increasing numbers of these animals that infest various regions of the earth.

Whether the sex ratio in the rat is dependent in any degree on the action of genetic factors cannot be determined from an analysis of the ratios as given in the present paper. The ratios as given for the extracted strains, which were derived from a cross between albino females and wild Norway males, are, on the whole, in accord with those for the albino strain. To ascertain whether genetic factors are involved it would be necessary to consider separately the ratios for a large number of individuals in the F_1 and F_2 generations of hybrid strains. Material is already at hand for such an analysis, which will be made later. From the evidence at present

available it would appear that the sex ratio is one of those
characteristics of a race that depends on a number of inter-
acting factors; some of which may be genetic, others physio-
logical and environmental.

Undoubtedly one of the physiological factors involved in
determining the sex ratio in the rat is the age of the mother
at the time of conception. As the ratios given in table 8 are
based on data for litter groups, they do not show the exact
relation of the age of the mother to the sex ratio in the young,
since some females begin breeding at a very early age and
may cast a litter every month during the early part of their
reproductive life, while other females begin breeding at a late
period and cast litters only at intervals of two or three
months. The advance in the litter series necessarily means
an advance in the age of the mother, and some idea of the
effect of the mother's age on the sex ratio in the young can
be obtained from the series of ratios given in the fifth column
of table 8. These ratios were calculated from the combined
data for all strains except the Norways.

Young female rats, i.e., those three to seven months old,
apparently tend to produce offspring in which there is an ap-
proximately equal proportion of the sexes, since the sex ratios
for the first two litter groups in the fifth column of table 8
show only 103 males to 100 females. The relative number of
male young increases with the age of the mother up to the
time that the latter is twelve to fourteen months old—the
period when the ninth and tenth litters are cast, as a rule.
An abrupt and striking change appears in the sex ratio in
the young at this point; female young tending to predominate
in all subsequent litters of the series.

The difference between the sex ratios for the first five litter
group in the fifth column of table 8 are not significant when
judged by their probable errors. Between the ratio for the
fifth group and that for the sixth group, however, the differ-
ence of 66.6 is over five times its probable error of ± 12.9,
thus indicating the action of some factor, as yet unknown,
that causes a marked decrease in the number of male offspring

cast by females approaching the menopause. The maximum sex ratio in the young is attained at a time when the mothers have passed the zenith of their reproductive activity and fertility is waning, as is shown by the marked decrease in litter production and in litter size. It is possible that the factor involved in the change in the sex ratio which comes at this time causes differential mortality in the sexes among the fetal young. If so, it acts at an early period of gestation and the dead fetuses are absorbed in situ, since there is no marked increase in the number of stillborn young at this period if the mothers are in good physical condition. Males are largely in excess of females among stillborn young regardless of the age of the mother (King, '21).

Since the Norway females, as a rule, did not begin breeding until they were about eight months old, the sex ratios given for the Norway strain are for the offspring of relatively old mothers. Data showing the sex ratio in litters of younger Norway mothers are few at present. Those obtained from later generations of this strain, where many females have begun breeding when from five to six months of age, indicate that the sex ratio is decidedly higher in the offspring of young mothers than in those of old ones. It appears, therefore, that in the Norway, as in other strains of rats, the sex ratio in the young decreases as the mothers approach the end of reproductive life.

SUMMARY

Data are given for the entire litter production of females in the following strains of rats: 1) stock Albinos; 2) extracted Albinos; 3) extracted Norways; 4) piebalds; 5) Norways. All of these strains of rats were reared in The Wistar Institute animal colony under similar conditions of environment and of nutrition. The data used in this study comprise 2063 litters containing 13,037 individuals.

Since the data for the first four strains are in close accord, the following general statements can be made for all of them. Exceptions found are noted in the text.

1. Females begin breeding when from three to five months of age, and reproductive activity extends over a period of from twelve to fifteen months, regardless of the coat color of the individual.

2. Although females may cast as many as fourteen litters, as a rule they produce only four or five litters during the course of reproductive life.

3. The second litter tends to be the largest of the series cast, containing an average of about 7.5 young. The number of young declines gradually in subsequent litters, dropping to an average of about 4.5 in the last few litters of the series.

4. In the entire series of litters cast by a large group of females the average number of young is about six. Litters larger than twelve are exceptional, and small litters (one to four young) are found usually when the mother is in poor physical condition or is at one extreme or the other of reproductive life.

5. The sex ratios in all strains show a pronounced, though not statistically important, trend with the advance in the litter series. The ratio tends to be low in the early litters cast; it rises slowly to a maximum, and drops to a very low point in litters cast at the approach of the menopause.

6. The sex ratios calculated from the total number of individuals in each of these four strains vary from 105.2 to 106.9 males to 100 females; these variations are not statistically important. For the total of 11,175 individuals the sex ratio is 105.7 males to 100 females.

Data for the Norway strain were obtained from litters cast by females in the first three generations born and reared in captivity. These data differ in many respects from those for the other strains under investigation.

Norway females, as a group, did not begin breeding until they were about eight months of age, and many of them did not cast a litter until they were a year old. The menopause appeared when the females were from eighteen to twenty-two months old.

In the Norway strain the first litter cast tended to be small, and the next two litters were the largest of the series. The average size of the litters gradually decreased to 4.8. For the entire series the average number of young was six.

The sex ratios obtained for the Norway strain are all low, and the trend is downward as the litter series advances. For the entire strain the sex ratio is 85.8 males to 100 females. This ratio is significantly lower than the ratio for any of the other strains.

Evidence given indicates that a low sex ratio is normal for the Norway strain, and that the higher sex ratio in the albino strain is due, in part at least, to the effects of domestication.

It appears, from the data given, that the age of the mother at the time of conception has an influence on the sex ratio in the young at birth. In the stock Albinos and in the extracted strains young mothers, i.e., those three to seven months old, tend to produce an equal number of male and female young. The number of male young increases up to the time that the mothers are from twelve to fourteen months old. In subsequent litters the number of female young is greatly increased. At all age periods Norway mothers tend to cast more female than male young, but toward the end of reproductive life the relative number of female young is greatly increased.

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NOTES ON REGENERATION IN TUBULARIA CROCEA

HELEN DEAN KING

NOTES ON REGENERATION IN TUBULARIA CROCEA.

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The following experiments on *Tubularia* (*Parypha*) *crocea* were made during the summer of 1903, at Woods Holl, Mass., while I was occupying a research room of the Carnegie Institution in the Marine Biological Laboratory. The work was done under the direction of Prof. T. H. Morgan to whom I am indebted for many helpful suggestions.

I. THE EFFECT OF THE EARLIER CLOSING OF ONE END OF A LONG PIECE OF THE STEM OF TUBULARIA.

In experimenting on the European hydroid *Tubularia mesembryanthemum*, Morgan (10) allowed the ends of long pieces of the stem to close and then, after an interval of from one to eight hours, he cut the pieces transversely through the middle region so that two new cut surfaces were exposed (Fig. 1, *B*, *C*). As a result, the aboral development of the proximal piece *CD*, was hastened. In many cases a polyp appeared on the aboral surface, *D*, as soon as did a polyp on the oral end, *C*, and in a few pieces a hydranth developed at *D* as early as did the hydranth on the distal end of the anterior piece, *AB*. This result is explained by Morgan as follows: "When a piece is cut in two in the middle one, two, three or more hours after its ends have closed, the influence of the oral end is temporarily removed, and the aboral end, which now has a start on the new oral end, may gain the ascendency and be the first to produce a polyp. Often, however, the polarity of the piece is sufficiently strong to give the precedence to the influences acting on the oral end. When the two influences are equally balanced, two hydranths may simultaneously develop."
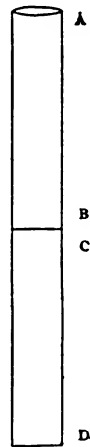
In repeating these experiments on the American hydroid,

FIG. 1.

Tubularia crocea, the hydranths already present at the anterior ends of the stems were removed by a transverse cut about 2 mm. behind the proximal circle of tentacles, and then pieces of stem varying in length from 30–40 mm. were cut off and the ends allowed to close. After an interval of from two to eight hours the stems were cut through the middle as in Fig. 1, *B*, *C*, thus producing a freshly cut surface (Fig. 1, *B*) at the aboral end of a distal piece, and also one at the oral end (Fig. 1, *C*) of a proximal piece. The results of this series of experiments are given in Table I. to IV. The first column gives the total number of individual stems operated upon; the second column shows the time that elapsed between the removal of the hydranth and the cutting of the stem through the middle; and in the following columns the results two, three, and four days after the operation are indicated. *Hy.* signifies the regeneration of a complete hydranth; *t. a.* indicates the formation of tentacle anlagen only; while *O* is used to indicate that no regeneration had taken place when the observations were made. The letters *A*, *B*, *C* and *D* refer to surfaces thus marked in Fig. 1; and the numbers in parentheses show the number of cases in which similar results were obtained.

TABLE I.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
6	2 hours.	$\begin{cases} A\ ...hy. & (6) \\ B\ ...\ O & (6) \end{cases}$ $\begin{cases} C....\begin{cases} t.\ a. & (3) \\ O & (3) \end{cases} \\ D...\ O & (6) \end{cases}$	$\begin{cases} A \\ B \end{cases}$ ......same. $\begin{cases} C....\begin{cases} hy. & (4) \\ t.\ a.\ (2) \end{cases} \\ D...\ \ t.\ a.\ (2) \\ \qquad O\ (4) \end{cases}$	$\begin{cases} A \\ B \end{cases}$ ......same. $\begin{cases} C......hy. & (6) \\ D...\begin{cases} hy. & (2) \\ t.\ a.\ (1) \\ O\ (3) \end{cases} \end{cases}$

Owing, probably, to differences in the temperature of the water in which they live, regeneration in *Tubularia crocea* is much slower than in *Tubularia mesembryanthemum*. In the former species a new hydranth rarely develops until two days after the removal of the old hydranth, while in the latter species a new hydranth frequently regenerates in the course of twenty-four hours. In all of the experiments in this series, as shown in the above table, a polyp formed on the oral end, *A*, of the anterior

piece, *AB*, before one developed on the oral end, *C*, of the prox-
imal piece, *CD*. This result might, of course, be expected as
the oral end, *A*, closed two hours before the end, *C*. In no case
did a polyp develop at the aboral end, *D*, of the proximal
piece as soon as one formed at the oral end, *C*. A start of only
two hours, however, is sufficient, in most cases, to cause the for-
mation of a hydranth at *D* before one develops at the aboral end,
B, of the distal piece, *AB*.

The results obtained when the intervals between the cuttings
were four, six and eight hours are given in Tables II. to IV.

TABLE II.

Number of Individuals.	Interval between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
10	4 hours.	$A \ldots \begin{cases} hy. & (6) \\ O & (4) \end{cases}$ $B \ldots \ O \ (10)$ $C \ldots \begin{cases} t.a. & (1) \\ O & (9) \end{cases}$ $D \ldots \ O \ (10)$	$A \ldots \begin{cases} hy. & (6) \\ t.a. & (4) \end{cases}$ $B \ldots \ O \ (10)$ $C \ldots \begin{cases} hy. & (2) \\ t.a. & (5) \\ O & (3) \end{cases}$ $D \ldots \begin{cases} t.a. & (1) \\ O & (9) \end{cases}$	$A \ldots \ hy. \ (10)$ $B \ldots \begin{cases} t.a. & (2) \\ O & (8) \end{cases}$ $C \ldots \begin{cases} hy. & (7) \\ t.a. & (3) \end{cases}$ $D \ldots \begin{cases} hy. & (1) \\ O & (9) \end{cases}$

TABLE III.

Number of Individuals.	Interval between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
8	6 hours.	$A \ldots \begin{cases} hy. & (3) \\ t.a. & (3) \\ O & (2) \end{cases}$ $B \ldots \ O \ (8)$ $C \ldots \begin{cases} hy. & (1) \\ O & (7) \end{cases}$ $D \ldots \ O \ (8)$	$A \ldots \begin{cases} hy. & (6) \\ t.a. & (2) \end{cases}$ $B \ldots \ O \ (8)$ $C \ldots \begin{cases} hy. & (5) \\ t.a. & (3) \end{cases}$ $D \ldots \begin{cases} t.a. & (4) \\ O & (4) \end{cases}$	$A \ldots \ hy. \ (8)$ $B \ldots \ O \ (8)$ $C \ldots \ hy. \ (8)$ $D \ldots \begin{cases} hy. & (4) \\ O & (4) \end{cases}$

TABLE IV.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
12	8 hours.	$A \ldots \begin{cases} hy. & (6) \\ t.a. & (4) \\ O & (3) \end{cases}$ $B \ldots \ O \ (12)$ $C \ldots \ O \ (12)$ $D \ldots \ O \ (12)$	$A \ldots hy. \ (12)$ $B \ldots O \ (12)$ $C \ldots \begin{cases} hy. & (10) \\ t.a. & (2) \end{cases}$ $D \ldots \begin{cases} hy. & (2) \\ t.a. & (3) \\ O & (7) \end{cases}$	$A \ldots hy. \ (12)$ $B \ldots O \ (12)$ $C \ldots hy \ (12)$ $D \ldots \begin{cases} hy. & (5) \\ O & (7) \end{cases}$

There is a great similarity in the results of these experiments. In all cases a hydranth formed on the oral end, *A*, of the anterior piece before one developed at any other cut surface, and the rate of development of a polyp from the aboral surface, *D*, was more rapid than that from the aboral surface, *B*, even in those cases in which *D* had only two hours start over *B*. In no case, however, did a polyp form at *D* before one developed at *C*, even in the experiments in which *D* closed eight hours before *C*. As was the case in the experiments made by Morgan, an interval of eight hours between the two cuttings has, apparently, no more effect on the regeneration than has an interval of only two hours. The formation of a polyp at the oral end, *C*, of the proximal piece, *CD*, does not prevent the early development of a polyp at the aboral end of the same piece, and in some cases there is only a few hours interval between the formation of the two hydranths. The earlier development of a hydranth at *A*, however, seems to check the formation of a hydranth at the aboral end, *B*, of the distal piece for some time, as in no case did a hydranth develop at *B* until five days after the experiment began. This difference in the rate of development at *D* and at *B* cannot be due to a difference in the lengths of the pieces, because, in making the experiments, the stems in all cases were cut as nearly as possible through the middle and any difference in the lengths of the anterior and of the proximal pieces would be too slight to have any appreciable influence on the result. The earlier closing of the aboral end, *D*, of the proximal piece, *CD*, evidently counterbalances to some extent the influence of the oral end, as suggested by Morgan. As a result, the development of a polyp at *D* is hastened somewhat, although in no case is a hydranth formed here before or as soon as one develops at the oral end of the piece.

The effect of the earlier closing of the aboral end of long pieces of the stem, in both *Tubularia mesembryanthemum* and in *Tubularia crocea*, is to hasten the development of the aboral surface. The influences that bring about this result are apparently not as strong in the latter species as in *Tubularia mesembryanthemum* where the aboral development may be hastened so much that polyps develop simultaneously at both ends of the piece.

This seeming difference between the two species may possibly be due to the fact that *Tubularia crocea*, which lives in cold water, regenerates very slowly and, therefore, comparatively slight differences in the rate of regeneration at the oral and aboral ends of the stem can be readily noted. *Tubularia mesembryanthemum*, on the other hand, lives in much warmer water and its regeneration takes place so quickly that it is difficult to detect slight differences in the rate of development of the polyps at the cut oral and aboral surfaces.

In a variation of the above experiment, a piece of silk thread was tied tightly around the stem about 2 mm. below the hydranth, and another piece was tied about 30–40 mm. below the first. Both ends of a long piece of stem were, therefore, closed at practically the same time in such a way that no regeneration was possible from either end of the piece. After the ends had been tied, the stem was cut transversely through the middle as in Fig. 1, *B*, *C*, in order to ascertain whether subsequent regeneration from the cut surfaces, *B*, and *C*, would be hastened in comparison with the rate of regeneration from similar surfaces of pieces of stem of the same length, cut at the same time, but not closed artificially at one end. The control pieces of stem were kept in the same dishes with those used in the experiment, and both sets, therefore, were under the same external conditions.

Eight long pieces of stem were used in this experiment. Two days after the operation, tentacle anlagen had appeared at the cut ends of all of the sixteen pieces, but they were not as well developed on the aboral end, *B*, of the anterior piece as they were on the oral surface, *C*, of the posterior piece. At this time there was no indication of any regeneration at the aboral surface of the anterior piece in the control set of stems, although in some cases complete hydranths, in other tentacle anlagen, were present on the oral end of the proximal pieces. On the third day after the operation, polyps were found on the oral end, *C*, of all of the proximal pieces, both in the control and in the tied stems. The development from the aboral surface, *B*, of the anterior pieces, however, did not keep pace with that at the oral end, *C*, of the proximal pieces, as at this time only two of the pieces of stem tied at one end had produced hydranths at the aboral sur-

face, *B*, the rest had, as yet, developed only tentacle anlagen ; in the control stems, no development from the aboral surface, *B*, had taken place in any case.

It is seen from the above experiments, that the development of a hydranth at the oral end of a piece of the stem of *Tubularia crocea* is not hastened by artificially closing the aboral end. Tying the oral end of a distal piece of the stem, however, hastens the development of the aboral end of the piece as compared with the development that takes place from the aboral surface of a piece of stem of similar length that is not closed at the oral end, as Driesch has shown. This result also agrees with that obtained by Loeb (7) in experiments in which he stuck the oral end of pieces of the stem of *Tubularia mesembryanthemum* in fine sand, leaving the other end freely surrounded by water. He found that "Durch Hemmung der Polypenbildung am oralen Ende kann man also die Polypenbildung am aboralen Ende beschleunigen."

In a third set of ten experiments, hydranth bearing stems about 30 mm. in length were removed from the colony and kept until a polyp formed on the cut aboral end. The time required for the development of these aboral polyps varied from three to five days in different cases. After all of the pieces had developed hydranths at the aboral end, each stem was cut transversely through the middle as in Fig. 2, *B*, *C*. The object of these experiments was to ascertain whether the presence of a hydranth at the aboral end, *D*, of the proximal piece, *CD*, would alter the polarity of the piece and thus prevent or retard the development of a hydranth at the oral end, *C*. The stems were cut through the middle region on June 19. Not until June 22 were there any indications of a development of a hydranth at the oral surface, *C*, and then only faint traces of tentacle anlagen were found in three stems. At this time, no development had taken place from the aboral surface, *B*, of any of the anterior pieces, *AB*. For control experiments, pieces of stem about 30 mm. in length were cut through the middle as in Fig. 1, *BC*, at the same time that the transverse cuts were made across the stems bearing a hydranth at each end, and on June 22, well developed hydranths were present at the oral end of all of the proximal pieces. On

June 23, three of the ten proximal pieces of stem bearing aboral hydranths had also developed hydranths at the oral end ; while the oral end of two other pieces of stem showed well developed

tentacle anlagen which developed into hydranths on the following day. No further changes took place in any of the pieces although they were kept for some ten days longer.

It is seen from the above experiments, that the presence of a hydranth at the aboral end of a piece of the stem of *Tubularia* delays, but does not prevent, the development of a hydranth at the oral end of the piece. This result cannot be due simply to the fact that the proximal end of the piece was closed by the presence of the aboral polyp, because, in the previous set of experiments, it was shown that closing the aboral end of a piece of stem by tying does not delay the development of a polyp at the oral end. It seems probable that the polarity of the piece was changed, for a time at least, by the presence of a hydranth at its aboral end and, therefore, the influences for hydranth formation at the freshly cut oral surface

FIG. 2.

were not strong enough to bring about the development of a polyp for some days.
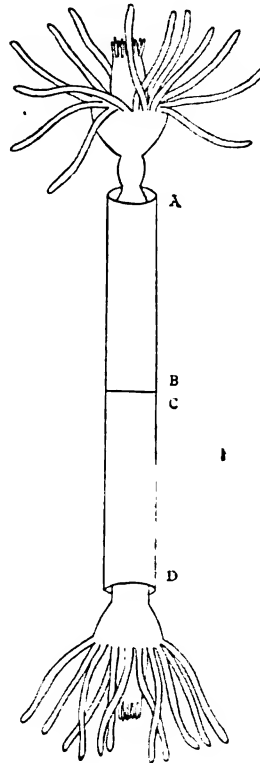
II. EXPERIMENTS ON BRANCHING STEMS.

The following series of experiments were made in order to ascertain whether the development of a hydranth on the oral end of a stem will influence the rate of development of a hydranth on the distal end of a long or of a short piece of a branch, and also to determine what conditions are necessary in order that the formation of a hydranth at the one place will prevent the formation of a hydranth at the other.

Series 1. — On June 24, twenty experiments were made in which a branch was cut off about 1 mm. from its origin in the stem, and then the anterior end of each stem was removed by a transverse cut leaving a piece from 10–20 mm. above the place of union with the branch (Fig. 3). In one case, a hydranth developed on the cut end of the branch two days after the operation, and at the same time a polyp also formed on the oral end of the main stem which in this instance was 20 mm. in length above the origin of the branch. In all other individuals at this time tentacle anlagen had formed at the oral end of the stem, but there was no indication of the development of a hydranth at the cut end of any of the branches. On June 27 hydranths were found at the oral end of all of the stems and also on the distal end of four branches; all the remaining branches had well developed tentacle anlagen excepting one which showed no signs of regeneration during the course of the week that the hydroids were kept. In this set of experiments, therefore, with the exception of the one case noted, regeneration of a hydranth took place at the distal end of the long stem before a polyp formed at the oral end of the short branch.

FIG. 3.

The results of this set of experiments might possibly be considered to be due to the fact that the longer piece exerted some kind of an influence over the shorter piece that would tend to alter the polarity of the shorter piece and thus retard development from its cut oral surface. That a larger piece of a hydrozoa can influence the polarity of a smaller piece is shown unquestionable in grafting experiments that I made on *Hydra viridis* (King, 6) in which the larger component of the graft either absorbed the smaller component or formed a permanent union with it. In the latter case, the polarity of the smaller piece was completely reversed, if necessary, in order that a structure might regenerate on its cut surface that would produce a normal polyp. Another factor that might, possibly, cause a delay in the development of a hydranth from the cut surface of the shorter piece is the length of the piece. Morgan has shown that in

branching stems a short piece of a branch or of a stem regenerates as does an isolated short piece, *i. e.*, the region of the tentacle anlagen is reduced and the rate of development of a hydranth is much slower than that of a hydranth from the cut end of a long piece of stem. Both of these factors may help to bring about the delay in the development of a hydranth from the oral end of the shorter piece in all of these experiments with branching stems in which there is a marked difference in the length of the stem and of the branch.

Series 2. — Seventeen branching stems from different colonies were cut so that the anterior portion of the stem above the place of union with the branch was only about 1 mm. in length, while the length of the branch varied in different cases from 8–25 mm. (Fig. 4). Two days after the operation, hydranths had developed at the cut

FIG. 4.

ends of thirteen branches, and well developed tentacle anlagen were found at the oral ends of the other four branches. No hydranths were found at this time at the oral end of any of the stems, and tentacle anlagen were only faintly defined in some few cases. On the next day, hydranths had developed at the distal end of all of the branches, but only five stems bore hydranths at the oral end. In this set of experiments the development of a hydranth took place more rapidly from the cut end of the long branch than from the oral end of the main stem.
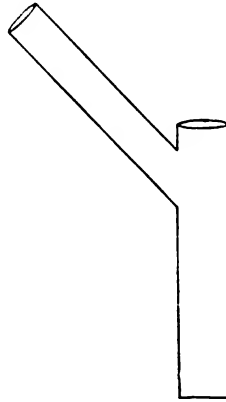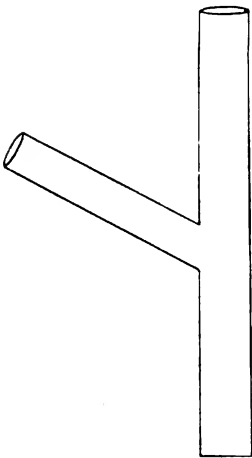
Series 3. — Twelve experiments were made in which the anterior end of the stem and the distal end of the branch were cut off so that the length of the branch and of the stem above the place

FIG. 5.

of union was practically the same, varying in different cases

from 8–20 mm. (Fig. 5). Two days after the operation, there were well developed hydranths on the ends of three branches and only tentacle anlagen on the oral ends of the corresponding stems. In two cases hydranths had developed on the end of the branch and also on the cut surface of the stem; while in the other cases only tentacle analgen were found, and they were equally well-developed on the branch and on the oral end of the stem. During the following two days, hydranths formed on all of the cut ends, sometimes the hydranth developed on the end of the stem before it did on the branch, and sometimes the hydranth appeared first on the branch. As a general result of this series of experiments it can be stated that hydranths develop at about the same rate when both the branch and the anterior portion of the stem are approximately the same length.

Series 4. — In the previous set of experiments both the branch and the anterior portion of the stem were of considerable length and both developed at about the same rate. In order to see if similar results would be obtained if the pieces were very short, fifteen experiments were made in which the branch and the stem were cut off about 1 mm. above their point of union (Fig. 6). When the hydroids were examined three days after the operation, hydranths were found on the oral end of the stem and not on the cut end of the branch in five cases; while in four hydroids, polyps had developed on the branch and not on the oral end of the stem; in the remaining six cases, hydranths were present at the distal end of both branch and stem.

FIG. 6.

In those cases in which one or the other cut surface failed to develop a hydranth, the cœnosarc appeared to be entirely with drawn from this part. When these stems were examined under the microscope, the streaming of granules in the interior cavity was visible only in the proximal part of the main stem and in the part of the stem or branch that had regenerated. In the cases in which hydranths formed at both cut surfaces, the streaming of granules was found in all parts of the stem and also in the branch.

In this set of experiments, regeneration seemed to take place

with equal rapidity from the cut oral surface of the branch and of the stem, as was the case in the experiments described under series 3. If the pieces above the place of union of the branch and stem are of approximately the same length, no matter how long or how short they may be, the influences for hydranth formation appear to be alike in both, and one piece has, seemingly, no effect on the other. Where the cœnosarc is withdrawn entirely from one part of the hydroid, as was noted in some few cases in the last set of experiments, no regeneration of the piece is possible.

In experimenting on *Tubularia crocea*, Morgan (9) found that if he cut off both the main stem and the branch a little above the place of union, the results varied considerably in different cases. In some instances he obtained the regeneration of a hydranth on the branch and not on the oral end of the stem; in other cases a polyp formed only at the oral end of the main stem; and in still other individuals regeneration took place from the cut surfaces of both branch and stem. It seems probable that the lack of uniformity in these results can be attributed to a difference in the relative lengths of the branch and of anterior portion of the stem above the point of insertion of the branch. It is, of course, impossible to cut the branch and stem at absolutely equal distances from the place of their union, and in those cases in which regeneration from one cut surface took place before it did from the other, there may have been just enough difference in the lengths of the pieces to bring about the earlier regeneration of a hydranth on the cut oral surface of the longer piece.

FIG. 7.

Series 5. — In twenty-eight cases the stem was cut off transversely just above the origin of the branch as shown in Fig. 7. The oral end of the branch was then removed leaving a piece, from 3 to 5 mm. in length, still attached to the stem. The object of these experiments was to see whether the regeneration of a hydranth at the oral end of the stem could be entirely prevented by this means. Two days after the operation, hydranths had developed at the cut end of the branch in nine of the hy-
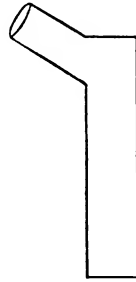
droids and well-developed tentacle anlagen were present on the
other branches ; there was no indication of a regeneration at the
oral end of any of the stems.   The next day all of the branches
bore hydranths, and in but one case had any regeneration taken
place at the oral end of the stem.   In this instance, the stem
extended about o. 5 mm. above the place of insertion of the
branch and a considerable amount of red pigment had collected
at its extreme oral end.   In the course of forty-eight hours more
a polyp formed on the oral end of this stem but no regeneration
took place at the oral end of any of the other stems, although
they were kept for over a week.

Series 6.—Sixteen experiments were made in which the branches
were cut off very close to their origin on the stem.   The oral
end of the stem was then removed leaving a piece about 5 mm.
in length above the origin of the branch, in order to see whether
the formation of a hydranth at the oral end of the stem would
prevent or merely delay the formation of a polyp at the place
where the branch was removed.   In all cases the wound in the
side of the stem healed over very quickly and, although the
hydroids were kept alive for a number of days, no regeneration
of any kind took place at the point of injury.

Series 7. — In ten cases the entire branch was removed from
the stem, but the old hydranth at the distal end of the stem was
not cut off.   The result was the same as in the previous set of
experiments, as the cut surface was very soon covered over and
no subsequent regeneration took place from it.

Series 8. — In sixteen cases where long pieces of stem bore
from two to four branches, the anterior end of the stem and the
apical end of each branch were removed by transverse cuts leav-
ing the lengths of the branches approximately the same as that
of the stem above the origin of the most anterior branch.   The
experiments were made to see if there is any difference in the
relative rate of regeneration of the anterior branches and of the
proximal ones.   There was no uniformity whatever in the results
of this set of experiments.   In some cases a hydranth regenerated
on a posterior branch before it did on the oral end of the main
stem ; and in other cases all of the branches produced hydranths
at the same time that one developed at the oral end of the stem.

III. THE REGENERATION OE SHORT PIECES OF THE STEM OF TUBULARIA.

It was first noted by Bickford (1), and later confirmed by Driesch (2) and by Morgan, that small pieces of the stem of *Tubularia* about 1 mm. in length are capable of regenerating.   In a recent paper, Hargitt (5) states that he was unable to obtain any regeneration from pieces of the stem of *Tubularia crocea* and of *Tubularia tenella* that were as much as 3–4 mm. in length.   This result was probably due to the poor condition of the stems when the experiments were made.   Small pieces of the stem of some other hydroids, do not appear to possess as great a power of regeneration as *Tubularia*, for Gast and Godlewski (4) have found that pieces of the stem of *Pennaria cavolinii* about 1 mm. in length never produce hydranths and pieces 2 mm. in length regenerate hydranths but rarely.   Bickford's experiments on small pieces of the stem of *Tubularia tenella* show that, in this species, regenerative processes are not restricted to any special region of the stem, and also that such short pieces tend to form one complete hydranth rather than to produce double abnormal structures.

In experimenting on *Tubularia mesembryanthemum*, Driesch (2) found that of 82 short pieces of stem, 5 formed a single proboscis, 26 formed a double proboscis, and the remaining 51 pieces produced hydranths.   These results agree with those of the earlier experiments of Bickford.   In a later paper, Driesch (3) states that at the oral end of the stem one seldom gets a whole hydranth, but usually a single or a double proboscis; from the middle zone hydranths usually develop; while from the aboral end of the stem, these structures are rarely produced. This difference in the kind of regeneration from the various parts of the stem Driesch attributes to the situation of the small piece in the original individual and to the different distribution of the hydranth-forming pigment in the cœnosarc of the different parts. Since Morgan and also Stevens (11–12) have proven the fallacy of the hypothesis of "red formative stuff" in *Tubularia*, this portion of Driesch's explanation is, of course, no longer tenable.

Morgan (8–10) has made an extended series of experiments with small pieces of the stem of both *Tubularia mesembryanthemum*

and of *Tubularia crocea*.   He finds, as did Driesch, that pieces
about 1 mm. in length from the region immediately behind the
old hydranth usually die, even when longer than pieces from a
more proximal region that regenerate.   When this distal region
does regenerate, it produces a greater number of single proboscides
than of other forms, a result that might be expected as this part
of the stem ordinarily goes into the proboscis of the new hydranth
when a long piece of stem is regenerating.

In another set of experiments, Morgan cut pieces of the stem
of *Tubularia mesembryanthemum* into a series of small pieces
about 1 mm. in length in order to observe the behavior of con-
secutive pieces from one stem and to compare the results with
those obtained from similar pieces cut from other stems.   His
tables do not show any very definite results although there seems
to be a certain similarity in the behavior of pieces of the same
stem, and the incomplete structures are found most frequently
at the distal end of the stem.

At the suggestion of Professor Morgan, I repeated his experi-
ments, using *Tubularia crocea*, in order to furnish more data from
which definite conclusions could be drawn.   In making the ex-
periments the old hydranths were removed and the distal part of
the stem was cut into consecutive pieces about 1 mm. in length.
The pieces were than laid in rows on the bottom of flat dishes
filled with fresh sea water.   For the sake of brevity the follow-
ing abbreviations are used in the tables given : hy. = complete
hydranth without any stalk ;   hy. + stalk = hydranth with a
short stalk that has been formed by a withdrawal of the cœno-
sarc from the perisarc ;   hy. + stem = hydranth with a stem
attached to the perisarc ; pb. = single proboscis ; d. pb = double
proboscis ; reprod. = reproductive organs.   The results tabulated
are from observations made three to four days after the operation.

TABLE V.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb. with no tentacles.	6	pb.
2	d. pb.	7	d. pb.
3	pb.	8	hy. + stem.
4	d. pb. + reprod.	9	d. pb.
5	pb.	10	d. pb.

TABLE VI.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	dead.
2	d. pb.	7	pb.
3	d. pb.	8	pb.
4	dead.	9	dead.
5	d. pb.	10	dead.

TABLE VII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	d. pb.	6	d. pb.
2	pb.	7	d. pb. + reprod.
3	pb.	8	pb.
4	pb.	9	hy. + stem.
5	hy.	10	pb.

TABLE VIII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb.
2	d. pb.	7	pb.
3	d. pb.	8	hy. + stalk.
4	pb.	9	pb.
5	pb. — reprod.	10	d. pb.

TABLE IX.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb. + reprod.
2	pb.	7	pb.
3	pb.	8	hy. — stem.
4	hy. — stem.	9	pb.
5	pb.	10	dead.

TABLE X.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	pb.
2	d. pb.	7	hy. + stem.
3	d. pb.	8	pb.
4	d. pb. + reprod.	9	hy.
5	pb.	10	dead.

As was the case in the experiments made by Morgan, the results for corresponding pieces of different stems are far from uniform, and it is not possible to determine what kind of a structure will be produced by a small piece from a given region of the stem. It is evident that the power to form either complete or double structures is present throughout the stem, and just what conditions are necessary to produce certain structures have not, as yet, been fully determined. .Morgan has suggested that possibly the factors in determining the kind of regeneration are (1) the smallness of the piece, (2) the differences in the region of the original stem from which the pieces came (this factor had been previously suggested by Driesch), (3) the age of the piece, as the younger the stem the more likely it would be to form incomplete structures.

According to this set of experiments short pieces, no matter from what part of the stem they are taken, are more liable to produce proboscides than to form hydranths. When the latter structures appear they are usually produced by the more proximal pieces of the stem, the distal end of the stem showing a great tendency to produce incomplete structures. These results are very similar to those obtained by Morgan on *Tubularia mesembryanthemum*.

In order to ascertain whether the double structures that are so often obtained in such experiments are produced because the small pieces of the stem are open at both ends and not because there is insufficient material in the piece to produce a complete hydranth, Morgan tied one end of a short piece with silk thread, and found that, under these conditions, double structures are never produced. Later he planted short pieces of stems in rows in sand so that one end was buried and the other freely surrounded by water. In two instances only was a double proboscis formed, in all other cases single structures, either incomplete or whole, more often the latter were produced.

In repeating these experiments of closing one end of a short piece of the stem of *Tubularia* in order to ascertain the effect on the kind of structure produced, the following method was used: Shallow, flat dishes were covered on the bottom with a layer of paraffine about one fourth of an inch in thickness, and then, with

the blunt end of a large needle about the diameter of a tubularian stem, rows of holes were made in the paraffine about 0.5 mm. in depth. The dishes were filled with water and the long pieces of stems were put in them and cut into consecutive pieces about 1 mm. in length. One end of each piece was then inserted in a hole that was just large enough to receive and hold it upright. This method has the advantage that the inserted end of the piece of stem is in contact with the paraffine and cannot become free if the experiment is properly done. The results of this series of experiments are summarized in the following tables in which the abbreviations used are the same as those previously employed.

TABLE XI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb.	6	pb.
3	pb. – reprod.	7	dead.
4	dead.	8	pb.

TABLE XII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy.	7	pb.
4	dead.	8	dead.

TABLE XIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	dead.	6	pb. + reprod.
3	d. pb.	7	pb.
4	dead.	8	pb.

TABLE XIV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	hy.	5	dead.
2	pb.	6	dead.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	dead.
3	pb. + reprod.	7	dead.
4	pb.	8	dead.

TABLE XVI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	pb.	7	pb.
4	hy. — stem.	8	dead.

TABLE XVII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb. + reprod.	6	dead.
3	pb.	7	dead.
4	pb. + reprod.	8	dead.

TABLE XVIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	dead.	5	dead.
2	pb.	6	hy. + stalk.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XIX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	dead.
3	hy. + stalk.	7	hy. + stem.
4	pb.	8	hy. + stem.

TABLE XX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy. + stem.	7	pb.
4	dead.	8	dead.

TABLE XXI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XXII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	dead.	6	hy. + stalk.
3	pb. + reprod.	7	pb.
4	pb.	8	dead.

The results of these experiments confirm those obtained by Morgan in every respect, as double structures were produced very rarely, only one being obtained in the entire series of experiments. There seemed to be no distinctive individual differences in the pieces of stem as regards the structures produced. In only one case (Table XIX.) were as many as three hydranths produced, while in the tables given by Morgan for *Tubularia mesembryanthemum*, whole series of pieces from the same stem produced hydranths.

If a comparison is made between the results shown in Tables XI. to XXII. and those shown in Tables V. to X., the most noticeable difference is that a very much greater number of double structures were produced when short pieces of the stem were lying on their sides during the process of regeneration. In both sets of experiments single proboscides were the structures most frequently produced, and very little individual difference could be detected in the stems regarding the kind of structure that they would tend to produce.

The development of small pieces of the stem of *Tubularia* standing on one end is considerably slower than that of similar pieces lying on one side. In the latter case, development takes place in about two days; while in the former case the various structures never appear under three days, and usually not under four or five days. Many pieces, usually those nearer the proxi-

mal end of the stem, die when the pieces stand on one end. This result may be due to the fact that the conditions under which regeneration takes place are not as favorable when the end of a small piece of stem is closed by contact with some foreign substance, as when the piece lies on one side and the cut ends are allowed to close in a normal manner.

BRYN MAWR COLLEGE,
Bryn Mawr, Pa., March 23, 1904.

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The Oögenesis of Bufo lentiginosus

By HELEN DEAN KING

THE OÖGENESIS OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

The present paper records the results of an investigation of the oögenesis of the American toad, Bufo lentiginosus, which was undertaken, primarily, in order to trace the history of the chromatin from the oögonia to the maturation period of the oöcytes and thus to complete my study of the chromatin behavior in the germ-cells of this amphibian. The work has necessarily involved a detailed study of the nucleoli, since these structures are closely associated with the chromatin at certain periods of development; and it has been extended to include an investigation of the yolk formation, as the material seemed especially favorable for this purpose.

This study was begun several years ago at Bryn Mawr College, but was laid aside for various reasons until this past year, when it was completed at the Biological Laboratory of the University of Pennsylvania, where I was holding a University Fellowship for Research in Zoölogy. I take this opportunity to express my obligations to Professor E. G. Conklin for many valuable suggestions during the course of my investigations.

I. MATERIAL AND METHODS.

Bufo lentiginosus is found very abundantly in the vicinity of Philadelphia; and, as the tadpoles are easily reared in the laboratory, several different series of preparations have been obtained consisting of larvæ killed at frequent intervals from the time of hatching until metamorphosis. These series give all stages in the development of the germ-cells up to the early growth period of the oöcyte. For the study of the later development of the ova, young toads with a body length of 1.5-5.5 cm. were collected at various times from June until

September.　In order to compare the development of the
ova in the young toad with that of the ova in the adult, por-
tions of the ovaries of mature females were preserved at
different times during the summer months.　As the eggs ap-
pear to develop along similar lines in all toads, ovaries of
young females were used principally for these investigations,
since in them the ova are more nearly uniform in size than
they are in the adult, and in a single section it is possible to
find a large number of eggs in practically the same stage of
development.

The very conflicting results that have been obtained by the
investigators who have studied the development of the germ-
cells in amphibians can doubtless be attributed, in part at
least, to the great diversity of ways in which the material
has been preserved.　Carnoy and Lebrun, who have studied
the germinal vesicle in the eggs of many different species of
amphibians, unhesitatingly recommend Gilson's fluid as the
best fixative for the amphibian egg.　I have not found that
this liquid gives a satisfactory fixation of the egg of Bufo,
as it usually causes a decided shrinkage of the nucleus and,
at certain stages, a distortion of the nuclear contents.　A
number of different fixing fluids have been tried during the
course of these investigations, among which may be mentioned
Zenker's fluid, corrosive-acetic (5 per cent acetic acid), cor-
rosive-formalin (Bouin's method), picro-acetic, Flemming's
solution, chromic-acetic, and Hermann's fluid.　Flemming's
solution (strong formula) is the best fixative for the oögonia
and the early growth stages of the oöcytes, although Zenker's
fluid and Hermann's fluid give very good results.　After the
yolk has formed Flemming's solution does not penetrate the
egg sufficiently well to give a satisfactory fixation.　For this
later period I have found that the chromic-acetic solution
recommended in a previous paper (King, 49) gives the best
preparations.　Corrosive-acetic acid is also a good fixative for
the egg at this period of its development, but it is especially
valuable for the maturation stages.　Corrosive-formalin and
picro-acetic do not give a satisfactory fixation of the egg of
Bufo at any stage of its development.

Of the great variety of combination stains that have been experimented with at different times in the hope that it might be possible to differentiate the chromatin from the nucleoli, safranin and gentian violet, used in the manner recommended by Hermann (39), proved to be by far the best. This stain is rather difficult to use, but when a satisfactory preparation has been made, all of the plasmosomes are stained a vivid red and stand out in sharp contrast to the chromatin which is a deep blue, while the structures which I have called "compound-nucleoli" are stained purple. Much of the material was stained with iron hæmatoxylin and orange G. This combination does not differentiate the nucleoli from the chromatin; but it gives such clear, sharp outlines that it is of great value in studying the early stages in the development of the oöcytes when the chromatin stains but faintly and cell boundaries are difficult to determine. Borax carmine combined with Lyon's blue, safranin followed by Lichtgrün, and Delafield's hæmatoxylin with orange G. also give good preparations, particularly of the later growth stages of the oöcytes.

II. THE PRIMORDIAL GERM-CELLS.

Owing to the large amount of yolk in the embryo and to the vagueness with which the cell boundaries are defined, it is impossible to trace the germ-cells in Bufo back to the segmentation stages of the egg, as has been done in other more favorable forms. Not until a tadpole is five or six days old and has attained a length of about 4 mm. can one point out definitely the group of cells that will develop into the genital ridge. At this stage of development the lateral plates of mesoderm (Fig. 1, L. M.) are well defined; the cells are small, with clear outlines, and they contain but comparatively little yolk. In the endoderm (Fig. 1, E.) on the contrary, the cells are very large; they are filled with yolk spherules which stain very deeply with iron hæmatoxylin, and their boundaries are irregular and difficult to determine. In the mid-dorsal region of the embryo there is usually found at this time a ridge of

cells (Fig. 1, G) which lies directly beneath the aorta (Ao.) and between the lateral mesodermal plates (L. M.). The cells of this ridge resemble the cells of endoderm in all respects and they are continuous with the endodermal cells which later form the lining of the digestive tract: there is no probability that these cells are of mesodermal origin, since they are always sharply marked off from the lateral mesodermal plates. When the tadpole is eight or nine days old this ridge of cells becomes separated from the endoderm, and it then forms a median cord of cells, the genital ridge, lying between the cardinal veins and supported by the mesentery (Fig. 2 G). The cells of the genital ridge are very conspicuous in sections at this time, as they still contain numerous large, deeply staining yolk spherules, although the neighboring cells have already absorbed the greater part of their yolk.

Many investigators who have studied the origin of the germ-cells in amphibians have asserted that these cells are derived from a germinal epithelium which is a modification of the peritoneal epithelium lining the body-cavity. This is the view advocated by Waldeyer (91), Semon (84), Hoffmann (44), Kolessnikow (55), Leydig (60), Spengel (86), Iwakawa (45), and more recently by Bouin (11). The last named investigator, however, admits the possibility that the first germ-cells in Rana are derived from endoderm, since in early stages of development they have all of the characteristics of the primitive endodermal cells. On the other hand, Nussbaum (73) maintains that in amphibians, and also in other vertebrates, the germ-cells are not derived from peritoneal epithelium, but that they are developed from undifferentiated embryonic cells which are set apart during early cleavage for this especial purpose. This theory has been supported by the researches of Woods (95) on Acanthias, and by Beard (7) on Raja batis. The latter investigator states: "The germ-cells may be regarded as unicellular organisms which pass one part of their life-history within a multicellular sterilized stock, the embryo or metazoön, formed by one of them at a definite period in the life-cycle."

In Bufo, as in the turtle and in the frog according to the investigations of Allen (1, 2), the germ-cells arise in connection with the endoderm.   Allen's recent account of the origin of the sex-cells in Rana pipiens agrees essentially with what I have found in Bufo.   I cannot be sure, however, that in Bufo the ridge of germ-cells is separated from the endoderm "by the approximation of the lateral plates of mesoderm," although many sections give this impression.   During this early period of development, when so many organs are rapidly being differentiated from embryonic tissue, it is impossible to tell exactly what forces or combination of forces are at work shifting the materials from one place to another.   It is possible, as Allen suggests, that the germ-cells themselves take an active part in the processes which separate them from the endoderm, since it is apparently only through their own activity that they reach their final position in the embryo.   Since Hertwig (41), Boveri (12), and others have traced the germ-cells back to segmentation stages, and Conklin (21, 22) has found various organ-forming substances in definite areas in the unsegmented egg, it seems meaningless to speak of organs as arising from any definite "germ-layer," although the convenience of such a starting point for the study of the development of any structure is obvious.   Owing to the character of the embryonic cells it is seemingly impossible to trace any organ in Bufo back to early cleavage stages, and the sex-cells are not clearly defined until the tadpole is about five days old. At this stage of development the germ-cells still retain their earlier embryonic character, and they are in contact with and closely resemble the endodermal cells.   Instead of asserting that the germ-cells in Bufo are endodermal in origin, it seems to me more in keeping with the results of the investigations on other more favorable forms to assume that these cells in Bufo are of like generation with the primitive endodermal cells and that both kinds of cells arise from neighboring regions of the unsegmented egg.   It may sometimes be possible to determine the organ-forming regions in the unsegmented egg of Bufo as Conklin has done in the egg of Cynthia.

When the genital ridge is first clearly marked off from the endoderm it occupies a median position between the cardinal veins and beneath the aorta (Fig. 2), as Bouin and Allen have stated is the case in Rana. If a section of the ridge in this stage of development is examined under high power one finds that it is composed of two distinct types of cells, one many times larger than the other (Fig. 3). The large cells, which are filled with yolk spherules and have vaguely defined boundaries, are the primordial germ-cells. The nuclei of these cells have the "mulberry" shape which La Valette St. George (78) discovered to be a characteristic of the nuclei in the spermatogonia of Salamandra, and they are usually crowded by the yolk spherules into one corner of the cell. The chromatin in these nuclei is in the form of minute, faintly staining granules which are distributed on linin threads or along the nuclear membrane. Each nucleus contains several rounded, deeply staining nucleoli of various sizes. Judging from their staining reactions most of these nucleoli are plasmosomes, and only one or two of the smaller ones are karyosomes. Scattered among these germ-cells, and frequently flattened against them, are numerous small cells which resemble in all respects the cells of the peritoneal epithelium from which they doubtless have been derived. These cells are very much smaller than the germ-cells; they contain no yolk and they have an elongated, deeply staining nucleus which is very large in proportion to the size of the cell. Doubtless these cells migrate into the genital ridge after the formation of the mesentery, since there are no cells of this type in the genital ridge at the stage of Fig. 1, and I have seen nothing that would indicate that they are derived from the germ-cells.

Bouin has stated that he finds in Rana temporaria transitional stages between peritoneal cells and primordial germ-cells, and he believes that before the metamorphosis of the tadpole new germ-cells are constantly arising from peritoneal cells. These observations have not been confirmed by Allen (2) in his study of the origin of the germ-cells in Rana pipiens, and in Bufo I can find no evidence that the germ-cells are

derived from peritoneal cells at any stage of development. The peritoneal cells in the genital ridge vary considerably in size and some may be nearly twice as large as others. In all cases, however, the cell contains comparatively little protoplasm and no yolk; while the nucleus maintains a characteristic appearance and stains very deeply, thus standing out in sharp contrast to the larger, more irregular, and more faintly staining nuclei of the germ-cells.

The development of the genital ridge proceeds from before backward. In a section of the anterior part of the ridge there are usually from 5-8 large germ-cells (Fig. 3), while in a more posterior section there are rarely more than three of these cells. In older tadpoles the difference in the rate of development of the different parts of the genital ridge is even more strongly marked, since the anterior portion of the ridge may have taken on its definite character as an ovary or a testis while the posterior portion remains in an apparently indifferent state.

When a tadpole is ten or eleven days old, the yolk spherules begin to disappear from the cells of the genital ridge and the structure of the germ-cells can then be more clearly seen (Fig. 4). At this time the germ-cells are more rounded than they were at an earlier period and, as they contain fewer and smaller yolk spherules, the polymorphic nucleus is usually found in the centre of the cell. With the exception of the large plasmosomes, the nuclear contents still show little capacity for staining either with plasma or with chromatin stains. By this time many peritoneal cells have become flattened against the germ-cells and have thus assumed the rôle of follicle cells. The boundaries of these follicle cells become very indistinct, and in many cases the cytoplasm seems to disappear entirely leaving the deeply staining nuclei in contact with the germ-cell.

In early stages of development the germ-cells are not always confined to the genital ridge. At the right, in Fig. 4, is a cell (Y) which lies considerably outside of the germinal area and directly under the Wolffian tubule; in Fig. 5, at the

left of the aorta are two germ-cells which lie above the level
of the genital ridge. Such germ-cells must eventually come
into the germinal area or degenerate, since cells of this char-
acter are never found outside of the genital ridge in later
stages of development. I have never found cells with the
characteristics of germ-cells in the mesoderm or in the ecto-
derm.

In a tadpole twelve to fourteen days old there is usually
found the beginning of a separation of the median genital
ridge into two ridges symmetrically placed one on each side
of the middle line (Fig. 5). This division of the genital ridge
is evidently brought about through the activity of the germ-
cells, although I have never been able to find any evidence
of amœboid movement in these cells. A longitudinal section
through a tadpole thirteen days old (Fig. 6) shows that, at the
time the genital ridge is dividing, the germinal area extends
from about the level of the liver nearly to the posterior end of
the body-cavity. When the division is completed the anterior
portion of each genital ridge contains from two to five germ-
cells (Fig. 7), while the middle and posterior portions rarely
contain more than one or two germ-cells (Fig. 8). Sections
through the posterior region of a genital ridge frequently con-
tain only the peritoneal cells (Fig. 9) which seem to be
crowding into the germinal area in increasing numbers at this
time.

The primordial germ-cells in the sex-gland of a tadpole
about to undergo metamorphosis are similar to those found
in the genital ridge at the stage of Fig. 4, except that they
contain only a small amount of yolk. After the greater
part of the yolk has been absorbed there is found in the cyto-
plasm of these cells a small, round, deeply staining, apparently
homogeneous body which is sometimes, though not invariably,
surrounded by a clear area (Fig. 8, V). This body, which I
shall call the vitelline body, divides previous to the cell mitosis
(Fig. 7, V), and one of these bodies is to be found subse-
quently in each of the daughter cells.

In addition to the vitelline body, there is found in the

cytoplasm of the germ-cells, usually close to the nucleus, a small centrosome which is surrounded by a rounded, granular attraction-sphere (Fig. 8, C). This centrosome divides very early in preparation for the cell mitosis, and, as shown in Fig. 7, it is sometimes possible to find a section of a cell which contains two centrosomes as well as two vitelline bodies. Such a section shows conclusively that the vitelline body is not derived from the centrosome and that there is no relation between these bodies. I have, as yet, no clue to the origin of the vitelline body which, as will be shown later, is undoubtedly concerned in the formation of yolk nuclei. A structure similar to the vitelline body is found in the cytoplasm of the spermatogonia of Bufo, and it can be traced directly to the spermatids where it gives rise to the acrosome of the mature spermatozoön. Since Meves (69), McGregor (63), and Broman (13) have found that the acrosome of the amphibian spermatozoön is derived from the idiozome, I suggested in a previous paper (King, 52) that the body in Bufo which forms the acrosome might possibly be derived "from a condensation of a portion of the attraction-sphere at an early period in the history of the primary spermatogonia." My study of the primordial germ-cells has not given any support to this hypothesis since, although this body is usually found near the attraction-sphere (Fig. 7), the two structures are clearly distinct at all times and there is not the slightest evidence that the former is derived from the latter. In its size and general appearance the vitelline body closely resembles the small nucleoli in the nuclei of the primordial germ-cells, but I have seen nothing that would indicate that it is of nucleolar origin. The later history of this structure in the ova strongly suggests that it is a secretion product of the cytoplasm formed, possibly under the influence of the nucleus, but not from nuclear material.

According to the investigations of Bouin, the increase in the number of germ-cells in Rana is brought about through a continuous process of transformation of peritoneal and mesenchyme cells into sex-cells, not by mitosis nor by direct division

of the germ-cells already present in the germinal area.   In
Bufo I have found that the multiplication of the germ-cells
is solely through mitotic division of the primordial cells
evolved from embryonic issue.   Although mitotic figures are
comparatively rare during the early stages of development
they are found very abundantly when the tadpole approaches
metamorphosis, and in a single section of the ovary of a toad
killed at this time one may find several cells that are preparing
to divide (Fig. 17, P).   Stages in the division of the primor-
dial germ-cells are shown in Figs. 10-14.   In the early pro-
phase of mitosis the chromatin forms a thick spireme which is
so much convoluted that it is impossible to determine whether
it is continuous or not.   This spireme is subsequently broken
into segments of various lengths (Fig. 10).   There are 24
of these segments, this being the number that is characteristic
of the somatic cells of the species.   Usually all of the nucleoli
have disappeared before the segments are formed, but some-
times, as shown in Fig. 10, Nu., a nucleolus will persist until
a much later period.   This would seem to indicate that the
nucleoli are not used in the formation of the chromosomes.
The chromatin segments shorten gradually and form broad,
V-shaped loops which can readily be arranged in pairs ac-
cording to their lengths (Figs. 11, 12).   In the metaphase
the chromosomes are arranged in a circle with the angle of
the V turned towards the centre of the spindle (Figs. 11, 13,
15); and, as they subsequently undergo a longitudinal divi-
sion, much narrower V-shaped chromosomes are found at the
spindle poles in the late anaphase (Fig. 14).

In sections of the ovary of a tadpole killed at the time of
metamorphosis germ-cells are frequently found which appear
to contain two or more separate nuclei (Figs. 15, 16, X).
Judging from these figures alone one might feel justified in
concluding that the germ-cells divide amitotically as well as
by mitosis.   I have never found a division of the cytoplasm
in any of the cases in which sections of the germ-cells contain
two or three nuclei, and in every instance the following or
preceding sections invariably show a connection between the
various unclei in the cell.   It is evidentfi therefore, that the

apparently multinucleated cells are not preparing to divide by amitosis. Their appearance is doubtless due to the fact that in sectioning the cells the polymorphic nuclei were cut in such a way as to completely separate two or more lobes. In my study of the germ-cells of Bufo I have never found a single instance where I could be sure that a cell was dividing amitotically; and I am convinced that this mode of division does not normally occur in any of the germ-cells of the ovary or of the testis.

By the time that a tadpole is sixteen to eighteen days old the anterior portion of each genital ridge has developed into a small rounded body, the so-called "Bidder's organ." The structure and development of this organ will form the subject of a separate paper and therefore no further mention of it will be made here, as it has seemingly nothing to do with the development of the ova.

Although sex is doubtless determined at a very early stage of development, the germ-cells of Bufo remain in an apparently indifferent condition for a long period, and it is not until the tadpole is about to undergo metamorphosis that its sex can be ascertained with any degree of certainty. Several investigators of amphibian oögenesis have stated that the presence of a central cavity in the genital ridge is the first characteristic by which the young ovary can be identified. In Bufo it is possible to distinguish the sexes at a somewhat earlier period of development by means of the arrangement of the cells in the more anterior portion of the sex-gland. In the young male the germ-cells are scattered evenly throughout the testis, each being surrounded by a number of follicle cells; in the young female the germ-cells have a definite arrangement around the outside of the ovary, while the centre is filled with peritoneal cells (Fig. 15). There is no central cavity in any part of the genital ridge at this time.

When the genital ridge has taken on the definite character of an ovary, some of the oögonia still contain a few small yolk spherules (Fig. 15), although all traces of yolk have long since disappeared from the other cells of the body. There is no ovarian wall at this time and the oögonia are surrounded

by follicle cells as in an earlier period. At a slightly later
stage of development (Fig. 16), the central part of the ovary
is no longer completely filled with peritoneal cells, but it con-
tains a number of intercellular spaces which later unite to form
one large cavity (Fig. 17). The central cavity in the ovary
of Bufo is not, therefore, a portion of the general body-cavity
which is brought into the ovary by a fold of peritoneal epithe-
lium as Hoffman has claimed is the case in Triton and in
various other amphibians, but it is the result of a fusion of
the many intercellular spaces which are produced by the rapid
increase in the size of the ovary. In Fig. 16 is shown the
beginning of the formation of the outer ovarian wall. At
the upper part of the ovary a number of peritoneal cells are
found with their nuclei flattened against the outer surface of
the germ-cells. The outlines of these cells become obliterated
and their cytoplasm forms a continuous layer over the
oögonia. At a slightly later stage (Fig. 17), many of the
peritoneal cells in the interior of the ovary become arranged
along the inner side of the oögonia to form the inner wall
of the ovary. In the young female as well as in the adult,
the ova develop between the two ovarian walls.

The small cells with deeply staining nuclei which are so
conspicuous in the ovary at the stages of Figs. 15-17 have
been called by various observers mesenchyme cells, peritoneal
cells, and follicle cells; while Bouin considers them to be
"petites cellules germinatives." In Bufo these cells are found
with the primordial germ-cells when the latter are first sepa-
rated from the endoderm at the stage of Fig. 3, and from their
general characteristics they are doubtless to be classed as
mesodermal cells. Occasionally these cells are found dividing
mitotically (Fig. 15, R); but division figures in them are
rare as compared with those that are found in the germ-cells.
The number of these cells increases enormously as the ovary
enlarges; and, since there is no evidence that they divide
amitotically, it is probable that there is a continuous migration
of cells from the mesentery through the ovary pedicle into
the ovary. Many of these cells later become the follicle cells
which are found around the egg as long as it remains in the

ovary; the others, as far as I can determine, are actively concerned in the formation of the ovarian walls, the cyst membranes and the zona pellucida.

According to the observations of Bouin there are fewer primordial germ-cells in a tadpole of Rana temporaria that is 33 mm. long than in one 20 mm. long. As the difference in numbers is considered too great to be attributed to individual variation, Bouin believes that a reduction in the number of primordial germ-cells is brought about at this stage of development through an expulsion of a large number of these cells from the ovary into the body-cavity. He calls this process "ponte d'ovules primordiaux," and he considers that it is analogous to that which occurs in the adult frog when the ripe eggs are expelled from the ovary. Bouin suggests that this process may take place so that "la glande, qui évolue dans le sens mâle, élimine les éléments qui seraient inutiles à son développement ultérieur." None of the other investigators who have worked on the development of the sex-glands in amphibians have described such a reduction in the number of primordial germ-cells and there is nothing similar to it to be found in Bufo. The expulsion of primordial germ-cells from the ovary is, therefore, either a process that is peculiar to Rana temporaria, or it is one which takes place so quickly in other species that it has escaped the attention of the investigators working in this field.

As the tadpole approaches metamorphosis, the ovary increases in size very rapidly and it usually appears lobed when examined in toto under a low power of the microscope. This lobed appearance of the young ovary furnishes a means by which it can be distinguished from the testis without making use of sections.

III. The Secondary Oögonia.

Soon after metamorphosis the primary oögonia give rise to a new generation of cells, the secondary oögonia, which are aggregated into cysts or "cell nests" that are arranged much

as are the primary oögonia shown in Fig. 17. The cells of a cyst are all descendants of one primary oögonium, and the cyst wall is formed evidently by the follicle cells which had previously surrounded the parent cell. The secondary oögonia are somewhat smaller than the primary oögonia, but they closely resemble them otherwise. They have a polymorphic nucleus containing a faintly staining reticulum and several plasmosomes. In the cytoplasm is a vitelline body (Fig. 18, V) and also a minute centrosome surrounded by a granular attraction-sphere (Fig. 18, C).

The cells of a cyst do not always divide simultaneously, and resting cells as well as cells in all stages of division may be found in the same cyst (Fig. 19). In the early prophase of mitosis a thick spireme is formed, as in the primary oögonia. This spireme breaks into segments (Fig. 19, S), presumably twenty-four, which condense into V-shaped chromosomes in the metaphase (Fig. 19, O). The spindle is the same shape as that found in the earlier generations of cells, and there are distinct centrosomes at the spindle poles which are devoid of any radiation (Fig. 19, O, R).

IV. The Development of the Oöcytes to the Synizesis Stage.

Considerable controversy has arisen among investigators regarding the origin of the oöcytes in the amphibian ovary since, at the period of the transformation of oögonia into oöcytes, cell and nuclear boundaries are frequently obscured and the cyst contents appear as a syncytium.

In his classic work on Bombinator igneus, Goette (35) states that in the young ovary the protoplasmic bodies of the central cells of a cyst fuse into a single mass which contains at first several separate nuclei; later the nuclei also fuse to form the mulberry shaped germinal vesicle of the egg. This view has been slightly modified by Bataillon (6), who concludes, from his observations on Rana and on Bufo, that after the fusion of the cytoplasmic bodies of the cells of a cyst one

of the nuclei wins the upper hand and subsequently absorbs all of the others.

Gemmil's (34) observations seem to indicate that "in der Regel geht aus einem Zellnest nur ein Ei hervor, und zwar durch directe Entwickelung aus einem der Elemente des Zell-nestes. Von den übrigen Elementen bilden sich einige wieder zurück und betheiligen sich an der Bildung der Granulosa, der Rest aber geht zu Grunde." According to Gemmil, there appears to be a struggle among the cells as to which shall form the ovum; space being the chief factor which de-cides the contest. The cell which lies in the centre of a cyst has seemingly the most room for development and this is the one which usually wins. The fate of the other cells depends upon how far they have differentiated before the one cell be-comes the ovum and so governs the rest. The cells which are least differentiated assume the rôle of granulosa cells. Those further developed cannot go backwards; they either have to become eggs or disintegrate. If the cyst happens to be larger than usual, as many as four of the cells may have room to develop into functional eggs. Since extra space is rarely ob-tained by the cyst, all of the cells which have passed a certain stage of development before the egg has formed are, as a rule, forced to disintegrate, and traces of the débris from these cells are to be found for some time in the protoplasm of the developing egg. Hoffmann's opinion regarding the origin of the egg in the Anura is similar to that of Gemmil, since he believes that one cell of a nest outstrips the others in development and forms the ovum while the others degenerate and become granulosa cells. Semon's observations lead to a similar conclusion.

Nussbaum and also Knappe (54) find a mulberry shaped nucleus in the primordial germ-cells, and they assert that this nucleus divides by amitosis into several small nuclei. One of these nuclei increases rapidly in size and becomes sur-rounded by the greater part of the cytoplasm of the cell, thus forming the egg; the other nuclei become arranged around the periphery of the egg to form the follicle epithelium. Ac-

cording to Eismond (27) an ovum may arise either from one
of the cells of a nest which has outstripped the others in
development, or from a fusion of all of the cells of a cyst. He
also considers that "la formation des nids n'était pas un anneau
indispensable dans le cycle de l'oögenèse, c'est-a-dire qu'en
même temps que la formation des nids du sens strict, se faisait
aussi la différenciation progressive des oöcytes directement
aux dépens des produits de la dernière division des oögonies,
comme cellules independantes." The conclusion that ova may
arise directly from oögonia accords with the view advanced in
1870 by Waldeyer (91) and supported later by the researches
of Balfour (4) on elasmobranchs.

Bouin has investigated the formation of the ova in much
greater detail than have any of the other workers on amphibian
oögenesis. He finds, as do other investigators, that secondary
oögonia are enclosed in cysts, and he states that all of the
oögonia in a cyst divide simultaneously. After several
divisions, the number of which he does not determine, the
character of the cells changes considerably and "oögonia of
transition" are formed. The latter are clearly defined cells
with rounded nuclei in which there are several chromatin
nucleoli, but no traces of a chromatin reticulum. This stage
is succeeded by one in which the nuclear membrane disap-
pears and the karyoplasm is separated from the cytoplasm
only by clear area. At a later stage of development granular
threads appear in the nucleus which are formed, doubtless, of
the minute chromatin granules scattered in the karyoplasm.
These threads increase in number very rapidly and form a
distinct chromatin reticulum, while a new nuclear membrane
encloses the nuclear contents. All the cells of a cyst develop
up to this stage, but later, owing to some unknown causes,
only a part of the cells continue their development as oöcytes;
the others degenerate and are either dissolved gradually or
devoured by the phagocytes. Degenerating cells never form
follicle cells but probably serve as nutriment for the victori-
ous oöcytes. The results of Bouin's investigations agree es-
sentially with those reached by Balfour in his study of

elasmobranchs. The latter investigator states that "some of the nuclei of each nest are converted into the nuclei of the permanent ova, others break down and are used as the pabulum at the expense of which the protoplasm of the young ovum grows."

Judging from the number of cells in a fully formed cyst, there are at most four or five generations of secondary oögonia in the ovary of Bufo. After the last oögonial division resting nuclei are formed, and the cyst is filled with small cells which appear much like that shown in Fig. 20. At this time cell and nuclear boundaries are very much more indistinct than in earlier stages, yet they can readily be made out in preparations fixed in Flemming's solution and stained with iron hæmatoxylin. If the material is properly preserved the cells never form a syncytium; nor is there any fusion of the nuclei, or any absorption by one nucleus of its less fortunate neighbors. Each cell in a cyst develops into an oöcyte, and, although I have examined a large number of cysts in this stage of development taken from many different individuals, I have yet to find a single instance in which there is a degeneration of any of the germ-cells in a cyst or any change of germ-cells into follicle cells. It seems very probable that the cells which several investigators have considered to be degenerating young oöcytes, were, in reality, cells in which the nuclei were in the condition shown in Fig. 25. This contracted state of the nuclear contents, to which McClung (62) has applied the term synizesis, is a definite constructive stage in the development of the young oöcyte of Bufo, and it is not due in any way to a degeneration of the nucleus or of the cell.

Owing to the crowded condition of the cells in a cyst the young oöcyte is more or less polygonal in outline. The nucleus is very large in proportion to the size of the cell, and it is invariably oval or slightly irregular, never possessing the polymorphic form characteristic of the nuclei in the earlier generations of cells. At this period the chromatin shows little capacity for staining and, as in the resting oögonia, it is in the form of minute granules which are either scattered along

the nuclear membrane or distributed on the linin fibres which form an irregular reticulum. The nucleus contains several deeply staining nucleoli of various sizes which are suspended in the meshwork of the reticulum or held against the nuclear membrane. In preparations stained with safranin and gentian violet the larger nucleoli invariably take the safranin while the rest of the nucleus is stained blue with the gentian violet, and these bodies must, therefore, be considered as plasmosomes; the very small nucleoli, which are found chiefly at the points of intersection of the linin threads, are karyosomes since they take the chromatin stain. In the cytoplasm, which stains very faintly and appears somewhat reticular, there is a vitelline body (Fig. 20, V); but I have not been able to find any traces of a centrosome or of an attraction-sphere in this or in any later period in the development of the oöcyte. As there are no centrosomes at the poles of the maturation spindle (King, 51), it seems probable that the egg centrosome disappears after the last oögonal division and that the attraction-spheres found at the poles of the segmentation spindle are formed in conjunction with the sperm-nucleus, probably under the influence of the centrosome imbedded in the substance of the sperm-head.

As the oöcyte enlarges its outline becomes more regular and much more distinct. The nucleus, which measures about 0.01 mm. in diameter at this time, soon assumes the rounded form which it retains up to the maturation period (Fig. 21), and its reticulum appears continuous and much more sharply defined than at an earlier period (Fig. 22). The number of nucleoli is not appreciably increased during the early growth period of the oöcyte.

V. Synizesis and Post-Synizesis Stages.

Although the stage in the development of the oöcyte shown in Fig. 22 is practically at the beginning of the growth period it corresponds, apparently, to the stage at the end of the growth period of the spermatocyte (King, 52; Fig. 15). In

both cases the nucleus contains a granular reticulum which appears to be continuous; and in both oöcyte and spermatocyte this stage is followed immediately by one in which there is a gradual condensation of the nuclear contents leading to synizesis (Fig. 25). The beginning of the process of condensation in the oöcyte is shown in Fig. 23, where the greater part of the chromatin reticulum is seen to be collected in the centre of the nucleus. In the following stage the contraction of the nuclear reticulum becomes more marked (Fig. 24), and eventually all of the nuclear contents forms a more or less rounded mass in the centre or at one side of the nucleus (Fig. 25). In favorable preparations the contraction figure is found to be composed of a tangled mass of exceedingly fine filaments in the meshes of which there are several round, apparently homogeneous bodies which are doubtless the plasmosomes: a number of the filaments run out from the central body and connect this structure to the nuclear membrane. At this stage it is impossible to follow in detail the changes that are taking place in the nucleus or to determine what relation the fine filaments bear to the nuclear reticulum of the earlier stage. The condensation of the nuclear contents in synizesis is not carried quite as far in the oöyctes of Bufo as it is in the spermatocytes where the contraction figure frequently appears as a rounded, apparently homogeneous mass connected by a few fine filaments to the nuclear wall (King, 52; Figs. 20-22).

In toads killed at the time of metamorphosis the ovaries contain large numbers of secondary oögonia and young oöcytes, although only a few of the latter have reached the synizesis stage at this time. Contraction figures are frequently met with in the ovaries of young toads killed about four weeks after their metamorphosis, and they are found very abundantly afterwards until the toad has attained a body length of about 1.5 cm. As I have already pointed out in the case of the spermatocytes of Bufo, I do not think it possible that the contraction figures are due to a bad preservation of the material as Janssens (47) has asserted is the case in Batracoseps atten-

uatus, since oöcytes with their nuclei in synizesis are found in
all parts of the ovary and frequently lie adjacent to oöcytes in
which the chromatin is in the form of a clearly defined con-
tinuous spireme (Fig. 22). Any method of fixation that
would cause such a decided distortion of the nuclear contents
in the one cell must of necessity have some effect on a neigh-
boring cell which is in but a slightly different stage of develop-
ment. In Bufo synizesis is not due to the degeneration of
certain cells as Kingsbury (53) has claimed is the case in
Desmagnathus fusca, since only in very rare instances are de-
generating eggs to be found in the ovaries of young toads.
Degenerating eggs, whether they are found in the ovaries of
young toads or in those of adults, are usually deeply pigmented
and they are invariably filled with phagocytes; they never
resemble in any way the oöcytes shown in Figs. 24-25.

Synizesis, which is a well recognized stage in the develop-
ment of the oöcytes and spermatocytes of many forms, has,
for the most part, been ignored by the investigators who have
worked on the germ-cells of amphibians, or its presence has
been considered as evidence of a degeneration of the cell.
Gemmil describes a stage in Pelobates fuscus in which the
nucleus of the young oöcyte contains a star-shaped mass of
chromatin which lies in the middle of a clear area and sends
out processes to the nuclear membrane. It is evident, from
the figures which Gemmil gives, that synizesis is the normal
stage in the development of the ova of this amphibian. Nuss-
baum figures condensation stages in the young germ-cells of
Rana fusca when they are enclosed in a cyst membrane. He
has, however, mistaken the order of sequence in the develop-
ment of the cells, as he considers that the contraction stage
preceded one in which the cell contains a mulberry-shaped
nucleus. Bataillon, Leydig, and Hoffman also mention the
appearance of contracting figures in the course of the normal
development of amphibian ova, although they venture no opin-
ion as to the significance of these bodies.

Bouin has entirely overlooked in Rana temporaria the young
oöcytes shown in my Figs. 20-22, and the earliest stage that he

figures as an oöcyte (Plate XII; Fig. 6), is about like that
of my Fig. 39.   He does not believe that synizesis is a nor-
mal stage in the development of the oöcytes of Rana, although
he figures contraction stages of the nuclei in cells which he
considers as oögonia that are not able to develop into oöcytes.
His description of the nucleus of one of these "degenerating"
cells is as follows: "On constate que les microsomes constitutifs
du réticulum chromatique se gonflent, se soudent les uns aux
autres, forment des amas irréguliers qui se colorent comme
les chromosomes des noyaux en mitose.   Ces amas peuvent
rester isolés dans l'aire nucléaire ou s'amalgamer en un bloc
chromatique de faibles dimensions."   The one figure which
Bouin gives of such nucleus (Plate XI; Fig. 15), shows the
synizesis stage in Rana which corresponds closely to that in
Bufo shown in Fig. 25; and many of his other figures show
post-synizesis stages comparable to those in Bufo (Plate XI;
Figs. 10, 11 : Plate XII; Figs. 2-5).

In a recent paper Lams (57) has given a description of the
stages in the early development of the oöcytes of Rana tempo-
raria which were overlooked by Bouin.   According to this
investigator the nuclear membrane does not disappear at any
time during the transition of the oögonia into the oöcytes.   In
the young oöcytes the chromatin filaments gradually condense
at one pole of the nucleus until they form a rounded, deeply
staining mass which appears much like that shown in my Fig.
25.   In post-synizesis stages this contracted mass resolves into
a system of filaments which subsequently divide longitudinally
and scatter throughout the nucleus.   This work of Lams, with
that of Bataillon and Leydig, furnishes conclusive evidence
that synizesis is a normal stage in the development of the
oöcytes of Rana.

It is unfortunate that the contracted condition of the nuclear
contents during synizesis prevents a detailed study of the
changes taking place in the chromatin at this time.   It is
evident that during synizesis the nuclear reticulum is no longer
continuous, and that it becomes broken up into a large number
of exceedingly fine filaments.   Some of these filaments appear

to be composed of a series of minute granules; others of delicate linin threads. As the plasmosomes can still be found during synizesis it is probable that they play no part in the changes taking place in the chromatin.

From the contraction figure shown in Fig. 25 there is evolved a long, apparently continuous, much convoluted spireme which is made up of a series of deeply staining chromatin granules distributed on a linin thread (Fig. 26). In the meshes of this spireme there are several nucleoli of various sizes, and there are also from one to five irregularly shaped, apparently homogeneous nuclear masses which are distributed along the nuclear membrane. These masses all stain intensely black with iron hæmatoxylin as does also the spireme. If, however, preparations have been satisfactorily stained with safranin and gentian violet the spireme is deep blue, the very small nucleoli appear red, while the large nucleoli and the masses against the nuclear membrane are purple, thus indicating that they are composite structures although they usually appear homogeneous at this time.

From the stage shown in Fig. 21 to that of Fig. 26 the oöcytes do not grow to any appreciable extent and the nuclei measure from 0.011-0.013 mm. in diameter. After synizesis there is a rapid increase in the amount of cytoplasm and in the size of the nucleus (Fig. 27). The chromatin spireme becomes more evenly distributed throughout the nuclear space, and it is noticeably thicker than at the stage of Fig. 26. In the succeeding stage the spireme begins to split longitudinally (Fig. 28). As the sister portions of the spireme are only about one-half of the thickness of the spireme at the stage of Fig. 27 it is evident that there is a true longitudinal division of the spireme at this time and not a folding together of chromatin filaments similar to that which occurs in the young oöcytes of the rabbit according to the investigations of von Winiwarter (93). At the stage of Fig. 29 the greater part of the spireme has divided and many of the sister threads have separated a considerable distance. When the splitting of the spireme has been completed the sister threads lie parallel, for the most

part, although they are not connected in any way. The threads do not present the clear cut, granular appearance of the spireme shown in Fig. 26, as they have a jagged outline and send out fine projections on either side.

There is absolutely no uniformity in the arrangement of the chromatin threads after the splitting of the spireme. At times the sister threads seem to lie close together throughout their whole extent (Fig. 30); again the sister portions of the spireme lie parallel for a short distance and then become widely scattered throughout the nucleus (Fig. 31); in rare cases, as shown in Fig. 32, the chromatin threads are as evenly distributed throughout the nucleus as they are at the stage shown in Fig. 27, and there is nothing except the size of the nucleus and the character of the threads to indicate that there has been a splitting of the spireme. I am very sure that such a condition of the chromatin as that shown in Fig. 32 could not have been brought about by a gradual lengthening of the spireme, since the great majority of nuclei intermediate in size between that of Fig. 27 and that of Fig. 32 appear similar to those shown in Figs. 28-31.

Soon after the stage of Fig. 30 the spireme breaks transversely, forming, in most cases, long double segments which vary considerably in length (Figs. 33, 34, 36, 37). The sister portions of the segments may lie parallel or they may be intertwined in various ways; they may be united at one or at both ends, forming a figure 8 or an oval ring; in other cases both ends of the segments are free and the threads cross in the form of an X or a Y. The condition of the chromatin threads shown in Figs. 30-34 is found in nuclei having a diameter of 0.015-0.02 mm.

I have tried to reconstruct a nucleus in the stage of development shown in Figs. 33-34, by placing together a series of camera drawings of all of the sections of the nucleus, in the hope that I might be able to determine by this means the total number of chromatin segments. Owing to the fact that the segments are of different lengths and that they are united in a great variety of ways, it has been very difficult to arrive at any

exact conclusion regarding their number. I believe, however, that the nucleus at this stage contains only the somatic number of chromosomes (24) which are usually arranged in twelve pairs. The question at once arises as to the value of the sister segments which form a pair. Is the splitting of the spireme shown in Figs. 28-30 a longitudinal division of chromosomes united end to end in the spireme or is it a separation of univalent chromosomes which had conjugated side by side? This question is very difficult to answer since it is impossible to determine what changes the chromatin undergoes during synizesis. As the nucleus apparently contains but twenty-four chromatin segments which in later stages of development are scattered throughout the nucleus and only occasionally found in pairs, I am inclined to the opinion that each of the sister segments represents an oögonial chromosome. The paired arrangement of the chromosomes at the stage of Figs. 33-34 strongly suggests that in the oöcytes of Bufo synapsis is coincident with synizesis as it is apparently in the spermatocytes; yet for various reasons, which will be given later, I am inclined to consider that synapsis does not occur until the beginning of the maturation period.

At the stage of Figs. 20-21 all of the young oöcytes in a cyst are approximately of the same size and in practically the same stage of development. As the synizesis period approaches the oöcyte which lies nearest the cavity of the ovary grows very rapidly and soon becomes several times the size of its neighbors. A section of a cyst with the oöcytes in this condition is shown in Fig. 37. The large cell bordering the cavity of the ovary has a diameter of 0.043 mm., and its nucleus measures 0.023 mm. in diameter. This oöcyte is surrounded by a number of follicle cells and its nucleus contains paired chromatin threads. The other cells in the cyst are very nearly of the same size; each measuring about 0.015 mm. in diameter and containing a nucleus measuring 0.01 mm. in diameter. These smaller oöcytes are in early post-synizesis stages of development, and they are not degenerating, as several investigators who have found a similar condition of the

cells of a cyst have claimed. The development of these cells is slower than that of the one cell simply because the size of the cyst is limited and there is no space for a more rapid growth.

Soon after the stage shown in Fig. 37 the cyst wall is ruptured, owing doubtless to the pressure of the growing oöcytes, and the larger cell becomes separated from the rest of the cyst and surrounded by a membrane which attaches it to the wall of the ovary. Inside of this membrane there are always found a number of elongated follicle cells which are undoubtedly concerned in the formation of the zona pellucida which later develops around the egg (Figs. 36, 39). As the other cells of the cyst enlarge each in turn becomes similarly attached to the ovarian wall. The cysts do not all develop at the same rate. In the ovaries of toads with a body length of 1.5 cm. one may find some cysts containing oögonia, others filled with young oöcytes in various stages of development up to that shown in Fig. 37, while in many cases the cysts have become disorganized and the ova are separately attached to the ovarian wall.

VI. The Nucleoli and the Later Growth Stages of the Oöcytes.

The irregular shaped masses of nuclear substance found against the nuclear membrane or in the meshes of the chromatin reticulum at the stage of Fig. 26 seem to increase in size as the nucleus grows and one of them usually becomes much larger than any of the others. These bodies appear homogeneous and stain black with iron hæmatoxylin or purple when the preparation is stained with safranin and gentian violet. When the nucleus has attained a diameter of about 0.025 mm. and the splitting of the spireme has been completed, numerous fine granular fibres are seen to project from these masses which do not stain quite as intensely as before (Fig. 34). At the next stage (Fig. 35) one obtains the first clue to the structure of these bodies. With the use of iron

hæmatoxylin the masses now appear grayish, and they are found to be composed of a meshwork of exceedingly fine fibres inclosing several darker homogeneous bodies. In preparations stained with safranin and gentian violet a much better differentiation is obtained. The meshwork of fibres invariably takes the blue of the gentian violet, while the rounded bodies in the interior, which are of various sizes, react differently towards the stain; the larger of these bodies, which are usually slightly irregular in outline, stain a reddish purple; the medium-sized ones, which are rounded and have a smooth outline, stain uniformly red, while the very small granules take the gentian violet. From the staining reactions of these masses, therefore, it is evident that they contain two different substances; fine fibres which are doubtless composed of chromatin not used for the chromosomes, and rounded bodies which are nucleoli.

For convenience in description I shall apply the term compound-nucleoli to the complex masses shown in Figs. 26-35 and also to the irregular nucleolar bodies shown in Figs. 39, 40, 43, 45, etc., reserving the term nucleoli for the smaller rounded bodies found in the interior of the larger masses at the stage of Figs. 35-36. The nucleoli which stain uniformly red with safranin will be called plasmosomes, while those that stain like chromatin will be considered karyosomes. In order to distinguish the chromatin of the chromosomes from that of the meshwork which forms part of the compound-nucleoli I shall refer to the former as "basichromatin" and to the latter as "oxychromatin." I am aware that these terms are not being used strictly in the sense in which they were introduced by Heidenhain (37), since both kinds of chromatin show the same color reactions with all methods of staining employed. Their use has been considered advisable here, however, in order to avoid the introduction of new terms.

At the stage in the resolution of a large compound-nucleolus shown in Fig. 36, the oxychromatin meshwork is much more clearly defined than at an earlier period and the threads are thicker and more granular. The number of nucleoli found in

the nucleus at this time greatly exceeds that found at any previous stage in the development of the oöcyte, and it is evident that a new formation of these bodies must take place during or soon after the synizesis stage. The compound-nucleoli in a nucleus do not resolve simultaneously. The larger masses are always the first to break up, and one or two of the smaller bodies may remain unchanged until the nucleus is twice the size of that shown in Fig. 36. Soon after the stage of Fig. 36 the meshwork of fibres becomes very loose and frequently breaks into several parts, while the nucleolar bodies begin to leave the fibres and scatter about the nucleus (Fig. 38). At the stage of Fig. 39 the resolution of the largest compound-nucleolus has been completed and the nucleus contains a number of nucleolar bodies of various sizes as well as several masses of tangled oxychromatin threads which are entirely separated from the nucleoli and easily distinguished from the chromosomes.

In his Fig. 15, Bataillon shows a portion of the nuclear contents of an ovarian egg of Rana which is very similar to one of the larger resolving masses shown in my Fig. 38. Bataillon believes that his figure shows the beginning of a connection between the chromatin filaments and the nucleoli, and he states that later the filaments disappear entirely, all of their substance going into the uncleoli. These results do not accord with what I have found in Bufo, since in the oöcytes of this amphibian the nucleolar bodies are preparing to leave the chromatin meshwork at the stage of Fig. 38, and chromatin filaments are to be found in all of the later growth stages of the ova.

At the stage of Figs. 33-34, the chromosomes stain somewhat more faintly than at an earlier period, and they are composed of a series of minute granules from which numerous fine fibres extend out a short distance on either side. In later stages these side projections become much more numerous and somewhat longer, and the chromosomes then come to have the feathery appearance shown in Fig. 39. At a later period the chromosomes stain so very faintly that in many cases they are to be found only with the aid of an immersion lens, yet

they retain the characteristic structure shown in Figs. 39, 40, 43, etc., and are therefore always to be distinguished from the oxychromatin threads. The chromosomes are never united with the nucleoli, although sometimes, as shown in Figs. 36, 37 and 39, a nucleolus is in contact with a chromatin thread; neither is there any connection between the basichromatin filaments and the oxychromatin threads. The latter stain much more intensely than the former and always appear to be composed of a series of rounded granules, they never have the feathery structure of the chromosomes. After the stage of Figs. 33-36, the chromosomes become widely distributed throughout the nucleus, and only a very few of them are found paired in later stages of development.

In a preliminary paper on the oögenesis of Triton, Janssens (46) gives a brief account of the changes taking place in the young oöcytes which seems to show that the behavior of the chromatin in the eggs of this amphibian is somewhat different from that I have found in Bufo. Janssens finds that synizesis occurs during the early growth period of the oöcyte, but he states that the reduced number of chromatin filaments appears shortly after this stage and that these filaments subsequently split longitudinally, the sister threads always remaining together in later development.

Carnoy and Lebrun (15-18; Lebrun, 58, 59) have written an elaborate series of memoirs dealing with the germinal vesicle and the polar bodies in various species of Batrachians. They have not studied the primordial germ-cells or the early growth stages of the oöcytes, and in every case their investigations begin with the young ovum at a stage about like that of my Fig. 27. Although the details of the developmental processes in the ova differ somewhat in the various species, Carnoy and Lebrun invariably find that, in the earliest stage which they have studied, the nucleus contains a chromatin filament which seems to be continuous. Later this filament disintegrates and gives rise to "primitive nucleoli" which move to the centre of the nucleus and there resolve into chromatin threads of various types. These chromatin threads soon break up into minute

granules from which new nucleoli develop to undergo the same series of changes as their predecessors. When the germinal vesicle disintegrates at the beginning of the maturation period certain of the nucleoli escape dissolution to form the twelve chromosomes which undergo a double longitudinal division in preparation for the maturation mitoses. At certain periods during the development of the ova, therefore, the nucleus contains no chromatin except that found in the nucleoli, and there is no "individuality" of the chromosomes or any reduction in the Weismannian sense during the maturation divisions. For Carnoy and Lebrun (16) the most important structures in the nucleus are the nucleoli. "Les nucléoles sont le chef-d'œuvre du noyau : ils représent le degré le plus élevé de l'organisation nucléinienne." In another paper (15) the statement is made that "les nucléoles sont des noyaux en miniature. Il renferme toujours un appareil nucléinien filamenteux plongé dans un plasma et logé dans une coque mince."

Carnoy and Lebrun give a large number of figures which are supposed to furnish evidence in support of their conclusions. They have, however, seemingly overlooked the important stages which give the clue to the nature of the "primitive nucleoli" and of their relation to the chromosomes (Figs. 28-39). In many of their figures they show feathery chromosomes similar to those shown in my Figs. 39-41, etc.; yet they consider that these chromosomes are products of the resolution of the nucleoli, as are also the granular threads which correspond to my oxychromatin filaments. The feathery chromosomes are often figured in pairs, the sister threads lying parallel or intertwined in various ways. Carnoy and Lebrun state that these paired filaments are not formed by a longitudinal or by a transverse division of a pre-existent nuclear element, but that they are either produced by a single filament folding back on itself and the parts separating, or they are two filaments which have been resolved from two nucleoli lying close together. Sections of nuclei are given by Carnoy and Lebrun which contain numerous nucleoli and no chromatin threads. Such figures are considered to prove conclusively that there

has been no continuation of the primitive filament. It is not difficult to find sections of the nuclei of the young ova of Bufo, particularly at the stages of Figs. 40-44, in which no chromosomes can be found. Such sections are possible because the chromosomes, which stain very faintly, are sometimes collected together at one side of the nucleus and sections passing through the centre of the nucleus show only granular karyoplasm, nucleoli, and possibly some of the oxychromatin threads. After the yolk has formed, many fixing fluids do not seem to penetrate the egg sufficiently well to preserve the delicate structure of the chromosomes. I have examined, under an oil immersion lens, every section of the nucleus of an egg preserved in Gilson's or Flemming's solution without finding the slightest trace of chromosomes; while in the nuclei of eggs from the same ovary that were fixed with chromic acetic or corrosive acetic the feathery chromosomes show very distinctly with a comparatively low magnification. I have never found a nucleus in which it was impossible to find the chromosomes, provided the egg had been properly preserved and stained.

In his earlier work on Axolotl, Fick (28) states that the nucleoli are independent structures which probably represent "eine Art Reservestoffbehälter." In a later paper on the ripening of the egg of Rana (29) he confirms the work of Carnoy and Lebrun and states that during the growth period of the oöcyte there are several generations of nucleoli which alternate with chromatin figures, consequently the continuity of the chromosomes is not maintained during this time. Fick considers that the nucleoli in the egg of Rana represent "eine Ruheform des Nucleins im Gegensatz zu den Chromatin-Figuren und Chromosomen, Formen in denen das Nuclein offenbar eine active Rolle spielt." Carnoy, Lebrun, Fick, and also Bataillon agree, therefore, with the conclusion reached many years ago by Schultze (83) from his study of the ripening of the egg of Rana, that the chromosomes "nicht aus einem präformirten Kerngerüst entsteht, sondern sich direkt aus den winzigen Keimkörperchen herausbildet."

The observations of other investigators of amphibian

oögenesis stand in direct contradiction to those cited above. Born (9, 10) states that in the egg of Triton the chromatin skein "sich aus dem Chromatingerüst des Ureies direkt herleitet." Although at one period of development the chromatin threads stain faintly and the chromatin substance can only rarely be distinguished from the granular karyoplasm, Born does not believe that the chromatin disappears or leaves the nucleus at this time, but that "sich dasselbe nur äusserst fein in der umgebenden Kerngrundsubstanz vertheilt habe." Later the chromatin threads are formed again, and they appear in pairs, lying parallel or closely intertwined as in Bufo. Born does not find that the nucleoli ever give rise to chromosomes, and while he ventures no conjecture as tō the function of these bodies he believes that they "stehen in Beziehung zum individuellen Zellleben nicht zur Fortpflanzung."

According to the observations of Jordan (48) on the newt, the chromatin threads "are distinctly traceable through the whole history of the germinal vesicle," although large chromatin granules break loose from the threads at various times and pass over into true nucleoli. Janssens has also asserted that in the egg of Triton the chromosomes persist throughout the entire growth period; but in this egg the chromosomes are entirely independent of the nucleoli.

Lubosch (61) has recently studied the history of the nucleoli in the ovarian egg of Triton with the avowed purpose of testing the work of Carnoy and Lebrun. His material was preserved and stained in a great variety of ways, and he concludes that many of Carnoy and Lebrun's results are due to the methods of technique which they employed. As Lubosch did not study the very young oöcytes, he ventures no opinion as to the origin of the primitive nucleoli. He states that nucleoli are formed periodically at the nuclear periphery, and that they then wander towards the centre of the nucleus where they undergo one of three modes of dissolution: (1) through vacuolization and subsequent differentiation into karyoplasm; (2) through distintegration into granules; (3) through transition into various sorts of chromatin filaments, some of which

are indistinguishable from the chromosomes at the time that
the latter are surrounded by a ring of nucleoli shortly before
the maturation period.   While Lubosch finds that the primi-
tive chromatin network becomes extraordinarily fine at certain
stages of development, he states that it never completely dis-
appears and that it is morphologically present in the ripening
egg, although it is in a finely divided form.

From the stage of Fig. 39 until that of Fig. 50, when the
nucleus has reached its maximum size and the nucleoli have
migrated to the centre preparatory to their final disintegra-
tion, the nuclei in the ova of Bufo contain an almost endless
variety of nucleolar figures.   In the nucleus shown in Fig.
39 the nucleolar bodies are of various sizes and they react
very differently towards the gentian violet and safranin stain.
The very small rounded nucleoli are karyosomes, since they
stain like the chromatin; the larger, rounded nucleoli may be
considered as plasmosomes, since they stain red and are not
connected in any way with the chromatin; the irregular body
marked X stains purple, and is a compound-nucleolus which
has not yet begun its resolution; while the small, slightly ir-
regular bodies are secondary compound-nucleoli which have
been evolved from the resolution of a large mass similar to
that shown in Fig. 35.   At Y is shown a nucleolar body which
is very similar to certain of the resolving nucleoli figured by
Carnoy and Lebrun.   This body is composed of a large, rounded
central plasmosome (staining uniformly red) which seems
to be giving off a number of small buds that also take the saf-
ranin.   The outer surface of this plasmosome appears some-
what irregular and stains purple because a number of oxy-
chromatin granules are attached to it.   This structure has
been produced, evidently, by the resolution of one of the com-
pound-nucleoli which contained only a comparatively small
amount of chromatin.   The pinching off of small plasmo-
somes from a larger mass is a very common phenomenon in
the ova of Bufo, and it is evidently one of the ways in which
the number of these bodies is increased.

Several small nucleoli are shown in Fig. 39 which are com-
posed of an outer ring of substance, evidently chromatin, since

it stains deep blue, surrounding a central portion which either stains very faintly or appears colorless; similar bodies are shown in Figs. 40, 41, 43, etc. At a later period the central portion of such nucleoli disappears, leaving only the chromatin ring. Subsequently the ring breaks at some point, thus becoming a crescent (Fig. 41), and it then disintegrates into small granules. Nucleoli of this character are probably derived from the oxychromatin of the larger compound-nucleoli, since they seem to be found most abundantly at the stages of Figs. 39-43. Similar nucleoli are figured by Carnoy and Lebrun and also by Lubosch.

. The oxychromatin threads produced by the resolution of the large compound nucleoli are massed together at the stage of Fig. 39; but they soon become scattered throughout the nucleus and are bent and twisted in a great variety of ways (Figs. 40-43). Occasionally, as shown in Figs. 40 and 43, two of these filaments lie parallel or cross each other in the form of an X. Such an arrangement is purely accidental, since the filaments never have any definite arrangement in the nucleus. In some cases oxychromatin threads seem to be united with nucleoli (Fig. 43); but as the nucleoli stain differently from the filaments, it is readily seen that there is no true connection between them.

Many of the figures given by Carnoy and Lebrun show granular chromatin filaments strikingly like those shown in my Figs. 40-43. These investigators consider that such filaments are derived from the substance of the nucleoli, and the contact of a nucleolus with a chromatin thread, as shown in my Fig. 43, is considered to be proof that the chromatin thread is being formed at the expense of the nucleolus. The feathery chromosomes are considered by Carnoy and Lebrun to be merely a special form of the filaments and in no way different from the others in origin or in fate. My observations do not admit of such an interpretation, since in Bufo the feathery chromosomes can be traced back to the continuous filament formed after synizesis (Fig. 26), while the oxychromatin threads are undoubtedly derived from the chromatin

which did not go into the spireme and they are always pro-
duced by the resolution of compound-nucleoli. Oxychromatin
filaments similar to those shown in Figs. 40-43 are figured in
Bouin's work on the oögenesis of Rana. Bouin considers that
these filaments are a part of the general chromatin of the egg,
and he does not distinguish them from the true chromosomes.

By means of a series of camera drawings of all of the sections
of nuclei in about the stage of development shown in Fig. 43,
I have endeavored to ascertain the number of oxychromatin
filaments and of chromosomes at this time. While the chromo-
somes appear to be twenty-four in every case, the number of
oxychromatin threads seems to vary from 20-50 in different
nuclei. This difference in the number of oxychromatin threads
in various cases can doubtless be attributed to the fact that
the compound-nucleoli from which the filaments are derived
vary in number and in size in different nuclei and that these
bodies do not all resolve at the same time.

Carnoy and Lebrun distinguish three distinct stages in the
development of amphibian oöcytes, and they state that there
are many generations of nucleoli which alternate with various
kinds of chromatin figures; the nucleus frequently containing
one kind of structure exclusive of the other. In Bufo I have
never found an oöcyte in a stage of development between that
shown in Fig. 38 and that of Fig. 50 in which the nucleus
did not contain nucleoli, chromosomes, and oxychromatin fila-
ments provided the egg had been satisfactorily preserved and
stained. As the ova grow the number of nucleoli increases;
but the number of chromosomes remains constant, and the
maximum number of oxychromatin filaments is found at the
stage of Figs. 40-43. After this time the oxychromatin
threads stain more faintly; the granules of which they are
composed gradually draw apart (Fig. 48), and finally become
scattered throughout the nucleus. Many of these minute
chromatin granules can still be found in the nucleus at the
beginning of the maturation period.

Although in Bufo there is no periodic resolution of nucle-
oli into chromatin threads followed by the development of a

new generation of nucleoli from chromatin granules, there is a constant formation of new nucleoli and a gradual dissolution of the old ones during the growth stages of the ova. The disintegration of small nucleoli composed of a ring of chromatin enclosing a plasmosome body, (Figs. 39-43) has already been described. The beginning of a dissolution of some of the larger nucleoli is shown in Fig. 42. In this nucleus many of the nucleoli are stained black with iron hæmatoxylin; others appear grayish, since they seem to have lost their capacity for staining intensely. The latter nucleoli are gradually dissolved in the karyoplasm; they are never resolved into chromatin threads. Although the process of dissolution usually involves the whole nucleolus, sometimes only a portion of it disappears leaving one or several small, rounded bodies (Fig. 40, X). It is possible that the small groups of nucleoli shown in Figs. 40 and 43 may have been formed in this manner.

As a rule the majority of the nucleolar masses which lie in the meshes of the chromatin spireme or against the nuclear membrane at the stage of Figs. 26-34, resolve into plasmosomes and oxychromatin filaments at about the time that the larger compound-nucleoli undergo their resolution (Figs. 35; 39, Y). These masses differ from the larger ones in that they contain a relatively greater quantity of plasmosome material and a much smaller amount of chromatin. One or two of these nucleolar bodies, rarely more, escape dissolution at the stage of Figs. 35-38 and appear in later stages as slightly irregular, round or oval structures which stain as uniformly, though in many cases not as intensely, as in an earlier period (Fig. 39, X). These bodies increase rapidly in size after the stage of Fig. 39, and they usually undergo a somewhat different mode of resolution from that of the other compound-nucleoli. At an early period in the resolution of these bodies the oxychromatin, which has been attached to the outer surface of the plasmosome substance (Fig. 40), breaks away and becomes scattered throughout the nucleus, being indistinguishable from the oxychromatin produced by the earlier resolutions of nucleolar masses. Sometimes all of the plasmosome

substance in these bodies forms one large rounded mass which contains either one large vacuole or a varying number of small ones (Fig. 40). Such a structure greatly resembles the large vacuolated nucleoli found by Carnoy and Lebrun and also by Leydig in the ova of various amphibians, and it is also very similar to the "principal nucleolus" described by Maréchal (67) in the selachian egg. In many cases the plasmosome substance in these bodies is divided, the greater part of it forming a large, rounded central mass which usually stains rather faintly and appears either homogeneous (Fig. 41, R) or vacuolated (Fig. 41, S), the remaining portion being broken up into a varying number of small, round, deeply staining bodies which are attached, for a time, to the outer surface of the larger mass and later separate from it to form small plasmosomes.

The large plasmosome bodies shown in Figs. 40, 41 and 51, disintegrate in various ways and at different times. In some cases they persist as rounded, vacuolated bodies until the germinal vesicle disintegrates at the beginning of the maturation period when they are slowly absorbed by the cytoplasm; in other cases they break open during the later growth stages of the ova (Fig. 51, b), and subsequently divide into several rounded portions which are gradually dissolved in the karyoplasm (Fig. 43). It is not uncommon to find these large plasmosome bodies budding off portions of their substance (Fig. 51, c, d); and it is probable that many of the nucleoli found at the stage of Figs. 48-50 have been formed in this way.

The central vacuole of these large plasmosomes frequently contains a number of nucleolini which may be separated or so joined together that they simulate a granular chromatin thread (Fig. 51, C, D). These nucleolini always stain like the plasmosome, and they are evidently granules which have broken away from the inner surface of the ground substance in a manner similar to that by which the small plasmosomes are budded off from the outer surface. It seems probable that Carnoy and Lebrun have in mind linear aggregations of

nucleolini when they state that chromatin filaments are often found in the interior of large nucleoli. As the result of an investigation of the structure of the nucleoli in many kinds of cells Montgomery (70) states: "I am forced to conclude that in all probability there are no skeins of chromatin lying in any metazoan nucleolus, since I have never found any evidence of chromatin in it in any metazoan cell." This statement may well be extended to include the nucleoli in the egg of Bufo, since in no case have I ever found chromatin filaments in the interior of rounded nucleoli, although they are often found wrapped around the exterior of a nucleolus (Figs. 38, 40, 43).

In preparations stained with safranin and gentian violet nucleolar bodies are sometimes found which are similar to the compound-nucleoli described above in size and general outline, although they have a very different structure as they are composed of a number of rounded plasmosomes imbedded in a mass of chromatin granules (Fig. 51, a). These bodies are compound-uncleoli containing a large amount of chromatin, which for some unknown reason did not undergo a resolution at the stage of Figs. 35-38.

Nuclei having a diameter of 0.04-0.08 mm. usually appear as in Figs. 39-43. They contain one or two large unresolved compound-nucleoli, a varying number of round plasmosomes and small karyosomes, together with numerous small compound-nucleoli which were set free from the large nucleolar masses at the stage of Figs. 35-38. These small compound-nucleoli, which I shall call secondary compound-nucleoli to distinguish them from the larger bodies, appear homogeneous and only slightly irregular at the stage of Figs. 39-43, and the greater number of them are masses at the side of the nucleus where the largest of the primary compound-nucleoli underwent a resolution at the stage of Figs. 35-36. In a slightly older egg metabolic processes occur which lead ultimately to the formation of yolk. These processes are accompanied by, if indeed they do not produce, a marked change in the appearance and in the behavior of the nucleolar bodies. At this stage of development (Fig. 44) there is a mass of very

irregular nucleolar bodies at one side of the nucleus which greatly resemble the structures figured by Carnoy and Lebrun as nucleoli resolving into their chromatin constituents. As the preparation from which Fig. 44 was drawn was stained with iron hæmatoxylin the nucleolar bodies appear homogeneous and stain very intensely. Their true character is shown only when preparations are stained with safranin and gentian violet. In such cases these fantastically shaped bodies are found to be composed of a number of rounded plasmosomes imbedded in a meshwork of oxychromatin granules (Fig. 45). The plasmosomes always appear homogeneous and they invariably take the safranin stain; the chromatin stains blue and it is always in the form of fine granules which may or may not be strung together in a filament. There is nothing to indicate that the chromatin in these structures is derived from the plasmosomes or vice versa. These peculiar bodies, which are found very abundantly in the oöcytes of toads with a body length of 4-5.5 cm., are unquestionably secondary compound-nucleoli which have increased considerably in size during the stages of Figs. 39-43 and are now resolving into their constituent parts, oxychromatin granules and plasmosomes. Soon after the stage shown in Fig. 44 these irregular masses break up, and for a short time the nucleus contains a number of plasmosomes surrounded by chromatin granules (Fig. 46). At a later period the oxychromatin granules separate from the plasmosomes and scatter thoughout the karyoplasm, and for the first time since before the synizesis stage the nucleus has all of its chromatin separated from the plasmosome substance.

As I was unable to obtain any young toads in the fall with a body length of over 5.5 cm., I have not been able to follow the later changes in the oöcytes in the ovaries of young females, and I have had to make use of the oöcytes developing in the ovaries of adults to complete my study of the oögenesis of Bufo. If adult females are killed soon after the breeding season in April or in May the ovaries are found to be filled with young ova, many of which contain nucleolar bodies simi-

lar to those shown in Fig. 44. A section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April is shown in Fig. 48. The nucleus has nearly attained its maximum size, and it is slightly oval, measuring 0.19 mm. by 0.22 mm. All of the irregularly shaped nucle-olar bodies found at the stage of Figs. 44-46 have disappeared and the nucleus contains a large number of round or oval nucleoli of various sizes which are entirely distinct from the chromatin threads. The smallest of the nucleoli, which stain like chromatin, have evidently been formed by a fusion of a number of the chromatin granules set free by the disintegra-tion of the oxychromatin threads. Most of the larger nucle-oli stain very intensely at this time and only a few of them show, by their lessened capacity for staining, that they have begun to dissolve. Since the majority of the nucleoli are derived from the resolution of the secondary compound-nucle-oli the greater number of these bodies are massed together in one part of the nucleus. A few oxychromatin filaments in the process of dissolution are still to be found in the nucleus at this time.

During early growth stages the nucleus occupies the centre of the ovum, but at or soon after the stage of Fig. 44 it begins to move towards the future animal pole of the egg. As at this time the nuclear membrane is usually somewhat irregular in outline, several investigators have maintained that the change in the position of the nucleus is brought about through amœboid movement. This explanation does not seem to me entirely satisfactory since in some cases the nuclear outline is perfectly regular when the nucleus is moving towards the upper hemisphere, and when irregularities in the nuclear out-line are found they are invariably distributed uniformly around the membrane, no matter by what means the egg has been pre-served.

At the time that the nucleus is changing its position the greater number of the nucleoli are massed together in one part of the nucleus (Figs. 44-48). I have examined many eggs in this stage of development and I have always found

that the greater number of the nucleoli lie in the part of the nucleus that is nearest the periphery of the egg. It hardly seems probable that this arrangement would be found so constantly if it had no significance. It seems to me a possibility, at least, that the accumulation of most of the nucleoli in one part of the nucleus may have something to do with the movement of the nucleus towards the periphery of the egg. This arrangement of the nucleoli strongly suggests also that the polarity of the egg is determined at or soon after synizesis, since the location of the largest of the compound-nucleoli, from which the greater number of secondary compound-nucleoli are derived, indicates the part of the nucleus which will be nearest the animal pole in the later growth stages of the oöcytes.

After the nucleus has reached its final position at the periphery of the egg there is a rearrangement of the nuclear contents so that the nucleoli become distributed fairly evenly around the nuclear periphery, while the chromosomes and the remains of the oxychromatin filaments are found in the centre of the nucleus (Fig. 49). Whether this arrangement is due to the activity of the nucleoli themselves, I have not been able to determine. These bodies always appear round and they never show processes similar to those found by Leydig and also by Eimer (26) and considered by these investigators to be the means through which the nucleoli change their position in the nucleus. It is at this stage of development shown in Fig. 49 that the nuclear membrane is most irregular in outline, and it is very probable that this irregularity is due to the close proximity of the nucleoli. As a rule all of the oxychromatin filaments have disintegrated at this time; the chromosomes can always be found in favorable preparations, although they stain very faintly.

Several investigators, among whom may be mentioned Will (92), Fick, and Leydig, maintain that at or before the stage of Fig. 49 nucleoli pass out of the nucleus into the cytoplasm where they either dissolve or take part in the formation of the yolk. Although I have examined a large number of eggs in

which there were many hundreds of nucleoli lying close to the nuclear membrane I have never found a single case in which a nucleolus was passing through the membrane into the cyto-plasm. At certain stages in the development of the ova there are a number of rounded bodies in the cytoplasm which greatly resemble nucleoli, and it is doubtless this similarity in ap-pearance that has led to the assumption that the cytoplasmic bodies are of nucleolar origin.

Soon after the stage of Fig. 49 the nucleoli leave their peripheral position and move towards the centre of the nucleus. Their arrangement at first is somewhat irregular (Fig. 50); but later, as shown in a previous paper (King, 49; Fig. 3), they form a closed ring which surrounds the chromosomes. This arrangement of the nucleoli in the ovarian egg previous to maturation seems to be characteristic of all amphibian eggs, as it has been noted by all of the observers who have studied this period in the development of the ova. All of the nucleoli have begun to disintegrate by the time that the germinal vesicle breaks down at the beginning of the maturation period. They first lose their capacity for staining and many of them become vacuolated. Later they break into small fragments which are absorbed by the cytoplasm.

Although this study of the ovarian egg of Bufo has shown that investigators of amphibian oögenesis have classed to-gether, under the general name of nucleoli, several different kinds of structures, it has not, unfortunately, disclosed the manner in which these bodies are formed or their function in the nucleus.

From the resting stage of the primary oögonium to the synizesis period in the oöcyte the nucleus of the germ-cells contains several rounded nucleoli which stain differently from the chromatin and there is not the slightest evidence that there is any genetic relation between them. During synizesis the nucleoli can still be distinguished from the chromatin (Fig. 25); but in early post-synizesis stages (Figs. 26-34) these bodies are contained in the amorphous masses of nuclear sub-stance (compound-nucleoli) left over after the formation of

the spireme, and they cannot be followed since the large masses stain very intensely and uniformly at this time. When the large compound-nucleoli resolve (Figs. 35-38) they liberate, with the secondary compound-nucleoli, many more of the rounded nucleoli, which I have called plasmosomes, than were found in the nucleus previous to synizesis. It is evident, therefore, that plasmosomes are being formed in the nucleus during early post-synizesis stages (Figs. 26-34). Since these nucleoli are formed only in the midst of oxychromatin granules it seems probable that the oxychromatin is concerned in some way with their formation; but the number and size of these bodies and the fact that they invariably stain differently from the chromatin seems to preclude the possibility that they are derived from chromatin substance as Flemming (30), van Bambeke (5), Macallum (65), Hertwig (43), Obst (7), Schockaert (79), Carnoy and Lebrun, Fick (29), and many others have maintained. The part played by the oxychromatin in the formation of the plasmosomes is obscured. There is no apparent decrease in the amount of this substance associated with an increase in the number and size of the plasmosomes during the early development of the oöcyte, and at the time that the oxychromatin filaments disintegrate the nucleus apparently contains its maximum number of plasmosomes (Fig. 48).

The plasmosomes seem to be of a plastic, semi-fluid consistency; they appear homogeneous until they begin to disintegrate, and in some instances they seem to be capable of increasing in size and of budding off portions of their substance. Judging from the appearance and behavior of these bodies and from the fact that their formation is associated with the rapid growth of the cell and with the formation of vitelline bodies in the cytoplasm, it seems probable that they are products of nuclear metabolism which are possibly depositors of nutritive substance that are to be used at a later period in the history of the cell. This is substantially the view advocated by Korschelt (56) and by Rhumbler (75). Montgomery (70-71) is one of the few investigators who believes that the nucleoli are of extranuclear origin. He states that in the egg of

nemerteans the nucleoli are first found closely applied to the inner surface of the nuclear membrane. "It would seem that the yolk is at first present in the cytoplasm in the form of a diffused, unstainable fluid; that a portion of it, that remaining in the cell body, later becomes segregated as, or chemically changed into yolk globules; and that another portion of it is taken into the nucleus and, after passing the nuclear membrane, is changed into nucleolar substance." Such an origin for the plasmosomes in the ova of Bufo seems unlikely since these bodies are not found close to the nuclear membrane until a late period in the development of the ova.

The large nucleolar masses found in the oöcyte at the stage of Figs. 26-34 correspond evidently to the "primary nucleoli" of Carnoy and Lebrun. I have shown that these bodies are complex structures composed of plasmosome material and of the chromatin which did not go into the formation of the chromosomes and that they later resolve into their constituent parts; they are never formed entirely of chromatin, as Carnoy and Lebrun maintain. The fantastically shaped nucleolar bodies found at the stage of Fig. 44 are similar in structure to the large compound-nucleoli shown in Figs. 26-34, and they too resolve into chromatin threads and plasmosomes. In the egg of Bufo there is never any connection between the nucleoli and the chromosomes. Only oxychromatin goes into the formation of the compound-nucleoli, and the oxychromatin filaments which are formed by the resolution of these bodies do not at once disintegrate to form a new generation of nucleoli but they gradually break up into minute granules which seem to be absorbed by the achromatic substance of the nucleus. It is impossible to determine whether these granules take any part in the formation of the chromosomes which are found on the maturation spindle.

VII. The Chromosomes.

At no period in the development of the oöcyte does the basichromatin disappear nor does it become condensed in the form of nucleoli, and the chromosomes can be traced continuously

from the time that they are first formed by the breaking of the spireme (Fig. 33) up to the stage when the germinal vesicle disintegrates in preparation for the maturation mitosis. At the time that the spireme divides longitudinally (Figs. 28-29) the chromatin filaments are found to be composed of a series of rounded granules from which a few fine fibres project on either side. When the spireme breaks into chromosomes the number of fine projections increases, evidently at the expense of the chromatin granules (Figs. 33, 34, 36). By the time that the oöcyte has reached the stage of Fig. 39, the appearance of the chromosomes has changed considerably. The axial portion of the thread is now composed of minute, faintly staining granules, evidently formed by the breaking up of the larger ones, and the fine projections from the sides are longer and more numerous than at an earlier period. The chromosomes, of which there are undoubtedly twenty-four, thus come to have the feathery appearance that characterizes them from this time until the beginning of the maturation period, and they greatly resemble the filamentous chromosomes found by Rückert (76, 77) in the selachian egg and by Born in the egg of Triton. After the stage of Fig. 39 the chromosomes stain very faintly, since the greater part of their substance seems to be in the form of fine fibres as it was during the synizesis period; they can always be found, however, if the egg has been properly preserved and stained. When the germinal vesicle is about to disintegrate the chromosomes lose their filamentous structure and become greatly condensed, appearing as a single series of perfectly round granules (King, 49; Fig. 8).

The arrangement of the chromosomes in the young oöcyte depends, evidently, on the extent to which sister portions of the spireme have separated before the spireme breaks into segments. The transverse division of a spireme like that shown in Figs. 31 and 32 produces chromosomes that are scattered irregularly throughout the nucleus and only occasionally paired; while the division of a spireme similar to that shown in Fig. 30, gives a paired arrangement of all of the chromo-

somes (Fig. 33, 34). In the later growth stages of the oöcytes the chromosomes become widely distributed throughout the nucleus and they seem to have no definite arrangement, although it is not unusual to find two chromosomes paired as in Fig. 43, or two chromosomes crossed as in Fig. 40.

In a previous paper (King, 49) I have shown that, at the time the germinal vesicle is about to break down in preparation for the maturation mitoses, the twenty-four chromosomes come together forming twelve pairs. The chromosomes of a pair are of the same length, but there is considerable difference in the lengths of the chromosomes of the various pairs. At this time "two of the chromosomes may be united in the form of an X or Y, a single or double figure eight, or they may lie parallel for a part of their length and the ends intertwine in various ways." At a slightly later period the ends of each pair of chromosomes unite forming a closed ring. Immediately following this stage the chromosomes apparently break up into granules and, owing to the changes occurring in the nuclear substance preparatory to the formation of the first polar spindle, it is impossible to trace the chromatin for a short period. When the polar spindle forms a large number of rounded chromatin granules are found near it which soon fuse into several irregular clumps from which the reduced number of chromosomes (12) is formed.

It is unfortunate that the chromatin cannot be traced during the period of the formation of the first polar spindle since it thereby becomes impossible to identify the chromosomes of the first polar spindle with the chromosomes that are found in the oöcyte previous to the disintegration of the germinal vesicle. If, however, the chromosomes can maintain their individuality in the resting nuclei of the oögonia and of the young oöcytes when the chromatin is in the form of minute granules which are scattered irregularly along a linin meshwork or distributed on the nuclear membrane, I see no reason why they should be considered to lose that individuality when they break up into granules at the beginning of the maturation period. After all it may be through the linin that the morpho-

logical continuity of the chromosomes is maintained; and it is very probable that there is a linin connection between the chromatin granules during the early maturation stages which I overlooked in my previous work. When opportunity offers I shall collect new material showing the formation of the first polar spindle in the egg of Bufo, in the hope that with some new method of fixation or of staining I can follow the history of the chromatin granules and thus trace the chromatin continuously from the early growth stages of the oöcytes through to the maturation spindle.

As far as I am aware, no investigator of amphibian oögenesis has as yet traced the chromosomes from the ovarian egg directly into the maturation spindle. Schultze, Carnoy and Lebrun, and Fick believe that the chromosomes of the polar spindles are derived from chromatin nucleoli which escape dissolution at the time that the germinal vesicle disintegrates. Other investigators have tacitly assumed that the chromosomes of the ovarian egg pass over into the maturation spindle and they have not given any figures of the critical stages.

Since there are two periods in the development of the egg of Bufo when it is impossible to follow the changes which the chromatin undergoes, it is a difficult matter to decide when and how synapsis occurs. The first period when the history of the chromatin is obscured is during the synizesis stage in the young oöcyte when the nuclear contents become massed together as shown in Fig. 25 and the chromatin appears in the form of exceedingly fine granular threads. This stage is succeeded by one in which the chromatin, which is to form the chromosomes, is in the form of an apparently continous spireme. Later this spireme splits longitudinally and then divides transversely forming the somatic number of separate segments. Assuming that the chromosomes maintain their individuality during synizesis, it is obviously impossible to determine how they were joined together in the spireme which is formed after the synizesis stage. If the chromosomes were joined end to end, then the splitting of the spireme shown

in Figs. 28-30 is a longitudinal division of univalent chromosomes. On this assumption synapsis is coincident with synizesis and, at the stage of Figs. 33-34, the nucleus contains twelve bivalent chromosomes which are divided longitudinally. This is the interpretation which Janssens has given to the early post-synizesis stages which he finds in the egg of Triton. The fact that in later growth stages the great majority of the chromosomes are not arranged in pairs makes this interpretation improbable for the egg of Bufo, since in the eggs of other forms when bivalent chromosomes divide longitudinally the parts remain together or are connected in some way.

If we assume that two chromosomes united side by side in the spireme, then the subsequent longitudinal splitting of the spireme is merly a separation of the chromosomes that were previously paired, and the transverse division of the spireme is the means by which the chromosomes are completely separated from each other. Synapsis, on this assumption, does not necessarily occur during synizesis, since the somatic number of chromosomes is evolved from the spireme. I am inclined to believe that synizesis in the egg of Bufo is a process by which the chromatin which bears the hereditary qualities and is to be used for the chromosomes of the maturation spindle is separated from the chromatin which has other uses in the cell. This would seem to bear out Gardiner's (33) contention that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter-cells, the other food for the cytoplasm." If this interpretation of synizesis is correct, the chromosomes must have been united in pairs in the spireme that was evolved from the synizesis stage, but synapsis does not take place until the beginning of the maturation period. This interpretation seems the more probable since Jordan failed to find a pairing of the chromosomes in the ovarian egg of the newt at any stage of development.

The second period when it is impossible to trace the history of the chromatin occurs just previous to the formation of the

first polar spindle when the twelve chromatin rings break into
granules which cannot be distinguished from the granular
achromatic substance of the nucleus. The twelve bivalent chro-
mosomes that are finally formed from the mass of chromatin
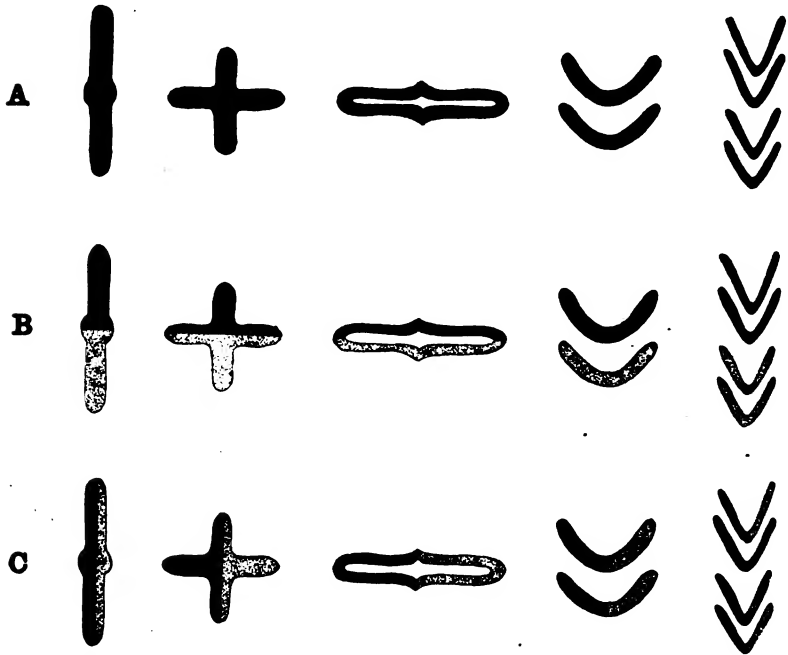
A.—Diagrams showing the changes in the shape of the chromosomes of
the first polar spindle in the egg of Bufo and the direction of the
maturation mitoses. In both mitoses the chromosomes are
divided longitudinally.

B.—Diagrams showing the character of the maturation mitoses if the
chromosomes were united end to end in synapsis. The first
division separates univalent chromosomes; the second is a longi-
tudinal division.

C.—Diagrams showing the character of the maturation mitoses if the
chromosomes conjugated side by side during synapsis. Both
mitoses divide the bivalent chromatin segments longitudinally.

granules that surround the polar spindle vary somewhat in
size and in shape. When they have become arranged on the
spindle they undergo considerable modification in form, and
the longitudinal axis of the chromosome at the time that the

first maturation mitosis occurs is the transverse axis of the chromosome at an earlier period (King, 51). In both maturation mitoses the bivalent chromosomes are divided longitudinally (Text-Figure I, A).

If synapsis occurs when the germinal vesicle disintegrates then the chromosomes must have united end to end, as shown in Text-Figure I, B, otherwise both of the maturation mitoses are equation divisions of bivalent chromatin segments and in neither division are univalent chromosomes separated (Text-Figure I, C). The investigations of Stevens (87, 88) have shown that in Sagitta synapsis takes place in the egg by a side by side conjugation of the chromosomes and in the spermatocytes by an end to end union. This is probably the plan that is followed in the germ-cells of Bufo if synapsis occurs in the egg during the synizesis period. I am inclined to the opinion that in the egg of Bufo, as in the spermatocyte, synapsis occurs shortly before the maturation mitoses and that the chromosomes are united end to end. On this assumption the first maturation division in the egg is a reduction division in the Weismannian sense since it separates univalent chromosomes, and the second division only is an equation division (Text-Figure I, B).

A. and K. E. Schreiner (80-82), who have recently examined the chromatin relations in the germ-cells of many different forms, conclude from their studies that in the germ-cells of *all* animals the chromosomes conjugate in pairs during synapsis, never end to end. This generalization finds an exception in the germ-cells of Bufo. In the spermatocytes of this amphibian synapsis occurs during the synizesis stage which immediately precedes the prophase of the first maturation mitosis, and in the prophase and metaphase of division the chromosomes behave in such a way that there seems no possibility of avoiding the conclusion that they were united end to end in synapsis. In the egg, as I have shown, it is not possible to determine when or how synapsis occurs; yet the evidence at my command seems to indicate that in synapsis the chromosomes are united end to end as they are in the spermatocytes.

VIII. The Formation of Yolk.

One of the most difficult problems met with in the study of amphibian oögenesis is that concerning the origin of the yolk. This problem includes not only a consideration of the origin and nature of the so-called "yolk-nuclei," but it also involves practically the whole theory of cell action since it cannot be supposed that the yolk formation takes place independently of nuclear activity. The anabolic processes taking place in the cell as a result of the interaction of the nucleus and the cytoplasm are as yet very imperfectly understood, and until we have obtained a clearer insight into the nuclear-cytoplasmic relations it will not be possible to solve the problem of yolk formation in an entirely satisfactory manner.

There is as great a diversity of opinion regarding the nature of the yolk-nucleus and the origin of the yolk in the amphibian egg as there is concerning the origin of the egg itself. The first observation regarding the presence of a yolk-nucleus in the amphibian egg were made by Cramer (23) in 1848. According to this investigator the cell body of the frog's egg contains a small granular ball which later spreads out in the form of a half-moon around the nucleus and gives off granules which develop into yolk spherules. In 1850 Carus (19) investigated the young egg of Rana temporaria and failed to find the granular ball described by Cramer. He states that the yolk first appears at the periphery of the egg in the form of single granules as it does in the egg of Alytes obstetricans according to the earlier observations of Vogt (90).

A few years later Thompson (89) wrote in regard to the presence of a yolk-nucleus in the frog's egg: "I have in general found it present, and think it more probable that it may be destined to form the external and larger corpuscles of the yolk, while the clearer part immediately surrounding the germinal vesicle may contribute to the production both of these and of the finer substance in which the germinal vesicle is found imbedded."

As Goette failed to find a yolk-nucleus either in the egg of Bombinator or of Bufo, Hertwig (40) is inclined to attach

but little morphological value to the rounded granular ball which he finds in the cytoplasm of the egg of Rana. "Mir scheint er einzig and allein mit der Bildung der Dottersubstanz in Beziehung zu stehen und eine eigenthümliche locale Ansammlung von Nährstoffen darzustellen." He suggests that the name "Dotterconcrement" would be more appropriate for this structure than "Dotterkern." Kolessnikow (55) mentions the presence of granular yolk-nuclei in the eggs of Rana temporaria, Rana esculenta, and Bufo variabilis, but he gives no opinion as to their origin or use.

Henneguy (38) finds a large granular mass, presumably a yolk-nucleus, in the egg of Rana, although he fails to find a similar body in the egg of Bufo vulgaris, Triton tæniatus, and Triton cristatus. Henneguy believes that wherever this body is found it is derived from the nucleolar substance of the nucleus and he ventures the interesting conjecture that "c'est un organe ancestral qui, avec les éléments nucléolaires de la vésicule germinative, correspond au macronucléus des Infusoires, le micronucléus étant représenté par le rôseau chromatique, prenant seul part aux phénomènes de fécondation."

Jordan finds a number of granular yolk-nuclei in the egg of the newt which he believes "arise from the cytoplasm and usually disintegrate in the cytoplasm." He is not sure whether these structures are of importance in the formation of yolk or not. Jordan's observations regarding the fate of these yolk-nuclei will be mentioned later.

The observations of several investigators seem to show that nuclear substance is used in the formation of yolk. In 1884, Will brought forward the view that in the ova of amphibians and of insects nucleoli leave the nucleus and migrate to the periphery of the egg where, as yolk-nuclei, they lose their sharp contour and break up into granules which become yolk spherules. Substantially the same view was advanced by Leydig in 1888 to account for the origin of the yolk in the egg of Triton. Leydig considers that the nucleoli arise in the nucleus "als Knotenpunkte in dem feinen Netz des Reticulums," and that they move to the periphery of the nucleus

where they "im losgelösten Zustande die Form und Natur kleiner Amöben zeigen." Subsequently they pass through the nuclear membrane into the cytoplasm and move to the periphery of the egg where they form groups of granules which develop into yolk.

Bataillon (6) also derives the yolk in the amphibian egg from the substance of the nucleus, but he believes that it is formed from the chromatin. "Des massules chromatiques issues de la vésicule germinative viennent donner dans le plasma ovulaire et à la périphérie d'abord, de véritables éléments cellulaires transitoires dont ils fournissent le noyau, et prendre part à la formation simultanié des tablettes vitellines et du pigment."

As a result of a study of the ovarian egg of Rana and of Necturus, Macallum (64) concludes that the peripheral chromatin nucleoli generate a substance which diffuses gradually through the nucleus into the cytoplasm. "I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm." Support for this view is furnished by the more recent cytological studies of Carnoy and Lebrun (15). These investigators state that the greater number of chromatin granules that are produced by the resolution of nucleoli are not used in the formation of a new generation of nucleoli, but that they are dissolved in the achromatin substance of the nucleus and transformed into nucleinic acid. This acid passes by osmosis through the nuclear membrane and is diffused through the cytoplasm. "Dans les plages formatrices, il rencontre les globulines de réserve imbibées d'eau, et se combine avec elles pour former la paranucléine d'abord, la vitelline en suite. . . . Nous considérons les plaques vitellines comme étant des produits de l'activité du noyau et du cytoplasme : celui-ci fournirait les globulines, le noyau, l'acide paranucléinique. Les vitellines sont, en effet, des paranucléo-albumines, c'est-a-dire des combinaisons de paranucléine avec un albumine qui est ici une globuline."

Jordan has observed in the newt appearances which might be interpreted as a migration of very minute granules from

the germinal vesicle into the cell body, and he also is inclined to the opinion that nucleus takes part in the formation of yolk. "One might suppose that granules from the germinal vesicle serve as starting points, centers of attraction or stimulation as it were, while the cytoplasm perhaps through the mediation of the yolk-nuclei, elaborates and supplies he requisite deutoplasmic material out of nutritive elements furnished it by the follicle cells."

Since it is seemingly impossible to harmonize these various observations regarding the yolk-nuclei and the yolk in the egg of amphibians, it can only be supposed, if these observations are correct, that the processes by which yolk is formed differ in various species. The details of these processes must, therefore, be worked out for each species separately, since there is no apparent similarity between them even in closely related forms.

In the egg of Bufo it is possible to trace the anlage of the yolk-nuclei back to the primordial germ-cells. As I have already stated, there is present in the cytoplasm of these cells a small, round, apparently homogeneous body which is sometimes, though not invariably, separated from the cytoplasm by a clear area (Fig. 8, V). This body colors very intensely with iron hæmatoxylin, and it always takes the safranin when sections are stained with safranin and gentian violet or with safranin and Lichtgrün. In preparations stained with Delafield's hæmatoxylin and orange G. this body is hardly discernible, since it takes the orange stain as does also the cytoplasm. Judging from its staining reactions this body is not chromatin; neither is it a centrosome, since the same section of the cell may show both of these structures (Fig. 7). I have not been able to determine the origin of this body owing to the fact that in very young tadpoles the large yolk plaques in the primodial germ-cells obscure the other cytoplasmic structures, while in older tadpoles, when the yolk is beginning to be absorbed, the small yolk granules show the same staining reactions as this body and therefore cannot be distinguished from it. Not until the tadpole is at least thir-

teen days old can this structure be distinguished with any degree of certainty. I shall apply the term "vitelline body" to this structure and also to other bodies of similar character which appear later in the cytoplasm, reserving the term, "yolk-nucleus" for the granular masses found in the cytoplasm at a much later period of development.

The vitelline body divides previous to each cell mitosis (Fig. 7, V). In sections of the ovaries of young toads this structure is found in the primary oögonia (Figs. 16-17), in the secondary oögonia (Figs. 18-19), in the young oöcytes at the critical period when the cell contents stain very faintly and cell boundaries and nuclear outlines are made out with difficulty (Fig. 20), and also during synizesis and early post-synizesis stages (Figs. 23-31).

In the early stages of development a cell rarely contains more than one vitelline body unless it is preparing to divide. During synizesis the vitelline body enlarges somewhat and at a slightly later period it becomes oval and then constricts through the middle so that it has the appearance of a dumb-bell (Fig. 47, a); subsequently it divides into two rounded parts (Fig. 47, b), which soon separate (Fig. 47, c). The vitelline bodies thus formed divide repeatedly, and by the time that the oöcyte has reached the stage of Figs. 36-39, its cytoplasm contains a considerable number of these bodies which vary greatly in size, although they all appear round and homogeneous. Sometimes at this stage a vitelline body is enclosed in a clear area which marks it off from the cytoplasm, but this is not a constant phenomenon. Occasionally a vitelline body does not divide in the manner described above, but it breaks into three small parts (Fig. 38, Y); in other cases division is unequal and one large and one small body are formed (Fig. 38. X). Since the vitelline bodies vary so greatly in size and are so widely scattered throughout the cytoplasm at the stage of Fig. 39, it seems very probable that some of them are newly formed secretion products of the cytoplasm which appear first as minute granules and gradually increase in size.

The vitelline bodies begin to increase in number before the resolution of the large compound-nucleoli and at a time when the nucleus contains but a very few plasmosomes: they are scattered throughout the cytoplasm, chiefly in the zone midway between the periphery of the egg and the nuclear membrane; and very few of them ever lie close to the nucleus. These facts would seem to preclude the possibility that the vitelline bodies are extruded nucleoli, although in their staining reactions and in their general appearance they are strikingly like these structures.

One of the reasons given by Will for considering the rounded bodies which he finds in the cytoplasm of the egg of Rana as extruded nucleoli is that he first finds these bodies in a light area close to the nucleus. Preparations of young ovarian eggs of Bufo that have been badly preserved frequently give the impression that nucleoli lie outside of the nucleus in a fluid space marked off from the cytoplasm. If such preparations are examined under an immersion lens, one finds that the light area which apparently surrounds the nucleus is, in reality, a portion of the nucleus itself, since the nuclear membrane is readily found where the clear area comes in contact with the cytoplasm. In such eggs, owing doubtless to the imperfect penetration of the fixing fluid, all of the more fluid portions of the nuclear substance seem to be collected at one side or around the periphery of the nucleus, while the granular achromatin and most of the nucleoli are massed together either in the middle of the nucleus or at one side of it. Projections from this mass sometimes extend across the fluid substance to the nuclear membrane and thus give the appearance of an amœboid nucleus without a nuclear wall. In nuclei of this character nucleoli are sometimes found stranded in the fluid substance and, under low magnification, they appear to lie in the cytoplasm. The clear area which separates the nucleus from the cytoplasm in many eggs is doubtless an artefact produced through the action of reagents: I do not think that it is present in the living egg.

The vitelline bodies are rarely found close to the periphery of the egg at the stages of Figs. 36-39, and I have seen noth-

ing that would indicate that follicle cells or their products enter the egg and produce these structures. As these bodies are not extruded nucleoli it is evident that they must be considered as secretion products of the cytoplasm itself. Since, as Bernard (8), Chittenden (20) and others have maintained, the nucleus is undoubtedly to be considered as an organ of constructive metabolism which "has controlling power over the metabolic processes in the cell, modifying and regulating the nutritive changes" (Chittenden), it is not to be supposed that the formation of the vitelline bodies in the cytoplasm takes place independently of nuclear activity. Although no substance can be seen to leave the nucleus at the time that the number of vitelline bodies is rapidly increasing, it is not improbable that a fluid, possibly an enzyme, passes from the nucleus into the cytoplasm and there causes the formation of these bodies. The same enzyme, acting in the nucleus itself, may be the cause of the formation of the plasmosomes; for these bodies are being produced in considerable numbers in the nucleus at the time that the formation of vitelline bodies is taking place most actively in the cytoplasm. On this assumption it is probable that the vitelline bodies "bear the same relation to the cytoplasm that the nucleoli do to the germinal vesicle," as Jordan has suggested. Whether the substance out of which the vitelline bodies are made is supplied entirely by the cytoplasm, or whether the follicle cells contribute material to the egg for their formation, I have not been able to determine. I have never found follicle cells inside of the egg, although they very frequently enter the cells of Bidder's organ. The function of the follicle cells seems to be to form the egg membranes during the early stages of development and, after the egg has left the ovary, to aid in the absorption of the follicle sacs (King, 50).

Several investigators of amphibian oögenesis, besides Will and Leydig, have found rounded bodies in the cytoplasm of the egg which are doubtless of the same nature as the vitelline bodies in the egg of Bufo. Hertwig (42) states that the small bodies which he finds in the cytoplasm of the egg of

Rana are composed of a hyaline substance and appear much like nucleoli, although they cannot be extruded nucleoli since nucleoli never wander into the cytoplasm. Born discovered small oval bodies near the nucleus in the cytoplasm of the egg of Triton which he hesitates to call yolk-nuclei since they never appear granular. Bataillon describes and figures the division of a small body lying in the cytoplasm of the egg of Rana which he considers to be a large nucleolus which has passed out of the germinal vesicle. He states that this body ordinarily disappears when the yolk is formed, and that he once saw it transformed into pigment.

Bodies similar to the vitelline bodies in the egg of Bufo have been found in the mammalian egg by von Winiwarter (94), Gurwitsch (36), and von Skrobansky (85). These bodies are present in the cytoplasm in addition to a granular yolk-nucleus. The latter structure, according to the researches of von Winiwarter and Gurwitsch, is homologous to the idiozome in the sperm-cells. The figures given by von Skrobansky of rounded bodies in the egg of the guinea pig are very similar to those shown in Figs. 36-38. Von Skrobansky states that these bodies increase in number as the egg develops and that they have a tendency to form in groups of two, three, or more which are often surrounded by a clear area. As their appearance is coincident with the disappearance of the yolk-nucleus, he suggests that the substance of the yolk-nucleus becomes differentiated into these rounded bodies, although it is not impossible that they are new differentiation products of the cytoplasm.

Small homogeneous bodies appearing like the vitelline bodies in the egg of Bufo have been found in the cytoplasm of the eggs of various arachnoids, myriapods, and vertebrates, and classed with large granular structures as yolk-nuclei. From the researches of Henneguy, Balbiani (3), and others, it is evident that the term yolk-nucleus has been used in a general way to cover a number of different structures in the cytoplasm, as the term nucleolus has been applied to a variety of structures in the nucleus.

When the toad has reached a body length of about 4 cm.
and the egg has a diameter of from 0.18-0.2 mm., there ap-
pears simultaneously in different parts of the cytoplasm a
number of irregular, granular masses which I shall call yolk-
nuclei since they are similar in appearance to the structures
described under this name by Foot (32), Henneguy, Jordan,
Calkins (14), and Munson (72). These yolk-nuclei arise as
rather small, irregular patches of granular substance that are
not sharply marked off from the surrounding cytoplasm.
There is at first no regular arrangement of these bodies; some
lie near the nucleus; others are found near the periphery of
the egg, while the majority lie in a zone midway between the
nuclear membrane and the outer boundary of the egg. When
sections are stained with any of the various combination stains
previously mentioned, these yolk-nuclei take the plasma stain
more deeply than does the cytoplasm and hence are easily
seen. It seems strange that such a keen observer as Goette
failed to find these bodies in the egg of Bombinator.

The manner in which these yolk-nuclei are formed is shown
in Fig. 46. Around one of the larger vitelline bodies there
appears a clear area, as if the vitelline body had in some way
caused a liquefaction of the surrounding cytoplasm (Fig. 46,
X). The substance of this area then becomes changed into an
irregular mass of minute granules which at first stain but
slightly darker than the cytoplasm. In some cases the vitel-
line body can be found in the centre of the yolk-nucleus (Fig.
46, Y), but as a rule it quickly loses its capacity for staining
and then disappears, evidently being used up in the formation
of the yolk-nucleus. As shown in Fig. 53, a yolk-nucleus
sometimes contains several vitelline bodies of various sizes
which stain as intensely as at the stages of Figs. 36-39. In
cases of this kind it is impossible to determine whether the
vitelline bodies in each yolk-nucleus are produced by the re-
peated division of the one vitelline body concerned in the
formation of the yolk nucleus, or whether several vitelline
bodies originally took part in the formation of a single yolk-
nucleus. I am inclined to the former view since the vitelline

bodies grow rapidly and divide very readily both in earlier and in later stages of development and they are rarely found in groups of more than three before the formation of the yolk-nuclei.

In eggs with a diameter of 0.25-0.3 mm. the yolk-nuclei are very conspicuous since they stain more intensely than at the stage of Fig. 46, and their number is much less than at an earlier period as several small granular masses fuse to form larger ones.   At this stage of development the yolk-nuclei come to have a definite arrangement in the cytoplasm, forming a more or less complete ring midway between the nucleus and the periphery of the egg (Fig. 44).   This is not an accidental arrangement found in a few eggs, but it is a constant phenomenon in eggs of a given size taken from different individuals and preserved and stained in different ways.   At this time the cytoplasm contains very few large vitelline bodies, most of these bodies having been used up in the formation of yolk-nuclei.

In the egg of the newt Jordan finds granular yolk-nuclei similar in appearance to those found in the egg of Bufo at the stage of Fig. 44.   Jordan states that these bodies appear about the time that the yolk is beginning to form at the periphery of the egg "from points of independent origin," and that there are never more than nine of these structures.   In the very young egg Jordan has found what appears to be "localized condensations of the cytoplasm and a consequent greater avidity for staining fluids," and he finds all gradations between these bodies and the granular yolk-nuclei.   From his observations Jordan concludes that "in the newt the yolk-nuclei always arise first as condensations of the cytoplasm and subsequently increase in size and complexity with the growth of the egg."   The figures given by Jordan do not show clearly the early development of the yolk-nuclei, although his Figs. 5, 10-12 are sufficiently detailed to indicate that the method by which the yolk-nuclei are formed in the egg of the newt is essentially the same as in the egg of Bufo.   The subsequent history of these bodies in the egg of the newt is similar to that

of the yolk-nuclei that are first formed in the egg of Bufo since, for a time, they lie in a zone half way between the germinal vesicle and the periphery of the egg and later draw near to the germinal vesicle where they gradually disintegrate. The only conjecture Jordan makes as to the probable function of the yolk-nuclei is that "they have a real physiological significance probably related to the construction of yolk."

The granular yolk-nuclei found by Foot in the egg of Allolobophora fœtida bear a striking resemblance to those found in the egg of Bufo (Cf. Foot's Figs. 4-5 with my Figs. 44 and 46). According to Foot the yolk-nuclei arise in contact with the nucleus, but, judging from their staining reactions, they are not derived from the chromatin as Calkins (14) maintains is the case in Lumbricus. As the yolk-nuclei increase in size they become broken up, and they are either scattered in patches throughout the cytoplasm or aggregated at the egg periphery. Foot concludes that these yolk-nuclei are formed of "archoplasm," and she traces them into the attraction-sphere, the fertilization cone, and the polar rings. Munson finds granular yolk-nuclei in the egg of Clemmys marmorata which are similar to the granular masses shown in Figs. 44 and 46. Munson states that these structures are formed of "a kind of metaplasm (or archoplasm) arising in the neighborhood of the germinal vesicle through the combined influence of the nucleus and cytoplasm. From the place of its formation, it diffuses or flows throughout the cytoplasm where it serves as a culture medium of the living substance of the egg; in other words, it serves as food. The true yolk-bodies are a secretion of the living substance of the cytoplasm."

Soon after the yolk-nuclei become arranged in the form of a ring there appears near the periphery of the egg a number of small, round, homogeneous bodies which stain intensely (Fig. 45). These bodies, as their subsequent history shows, are a new generation of vitelline bodies which are directly concerned with the formation of the yolk. From their peripheral position one might, perhaps, be inclined to think that the follicle cells are concerned in some way with the formation

of these bodies. I have never found any evidence that would support such an assumption. Since these vitelline bodies are formed some distance from the yolk-nuclei and are at first nearly uniform in size, it would seem as if they must be new secretion products of the cytoplasm. There is the possibility, however, that they are derived either from the few vitelline bodies that were left over after the formation of the yolk-nuclei or from the granular substance of which the yolk-nuclei are composed. The vitelline bodies increase very rapidly in number and in size, and many of them give rise to small granular yolk-nuclei, similar to those shown in Fig. 46, which always remain at the outer surface of the egg (Fig. 54).

While the new formation of vitelline bodies is taking place at the egg periphery, the ring of yolk-nuclei is gradually moving towards the centre of the egg and at the stage of Fig. 54 it closely encircles the germinal vesicle. In many cases yolk-nuclei seem to come in actual contact with the nuclear membrane. Whether there is a fusion between these bodies and the substance of the germinal vesicle, as Jordan is inclined to believe, I am not able to state. There is no noticeable increase in the size of the nucleus or any unusual change in the nuclear structure as one might expect to be the case were a considerable quantity of substance taken at this time into the germinal vesicle. After reaching the germinal vesicle the circle of yolk-nuclei stains less intensely and gradually disappears (Fig. 56). I am inclined to the opinion that their substance is dissolved in situ to take part later in the formation of the yolk spherules in this region of the egg. This view agrees substantially with that advanced in 1859 by Thompson and since advocated by Jordan.

As a rule the yolk-nuclei that are first formed have moved close to the germinal vesicle by the time that new yolk-nuclei are to be found at the periphery of the egg, and there is a cytoplasmic zone between them which is free from vitelline bodies or yolk-nuclei (Fig. 54). In exceptional cases, as shown in Fig. 53, the formation of the peripheral vitelline bodies and yolk-nuclei takes place even before the older yolk-

nuclei have become arranged in the form of a ring, and the
only portion of the cytoplasm which does not contain these
structures is that surrounding the germinal vesicle. As these
cases are found so infrequently I have not been able to de-
termine whether the yolk-nuclei later become arranged as
in Fig. 54, or whether all of them remain at the periphery of
the egg to take part in the formation of the yolk there.

In Bufo, as in other amphibians according to the investiga-
tions of Vogt, Goette, Schultze, Born, Iwakawa, Jordan, and
Dubnisson, yolk spherules first appear in the outer regions
of the cytoplasm and usually simultaneously in different parts
of the egg. There are apparently two methods by which the
first yolk spherules may be formed in the egg of Bufo. Both
of these methods sometimes take place in the same egg;
whether this is true for all eggs I am unable to say. The yolk
develops so rapidly that it is difficult to follow the processes
of its formation in any detail.

Soon after the stage of development shown in Fig. 54 a
varying number of small, oval bodies appear in the peripheral
yolk-nuclei (Fig. 56). These bodies, which stain somewhat
less intensely than the vitelline bodies, are yolk spherules which
are being formed, evidently, from the substance of the yolk-
nuclei. As the yolk spherules increase in number the granular
yolk-nuclei fade away and they have completely disappeared
by the time that the yolk forms a continuous layer around the
periphery of the egg.

In some cases certain of the vitelline bodies at the periphery
of the egg grow very large (Fig. 55). The outlines of these
bodies become irregular (Fig. 52, a), and subsequently they
break into from two to four rounded, homogeneous pieces
(Fig. 52, b) which in turn divide into smaller bodies (Fig. 52,
c-e). As a result of the repeated division of the one vitelline
body there is formed a mass of small oval bodies (Fig. 52, f)
which are of the same size and shape as the small yolk spher-
ules, although at first they stain more deeply than the yolk
spherules and can therefore readily be distinguished from
them. Later these bodies stain less intensely and seem to pass

over into yolk spherules. It is very probable that the aggregations of small yolk spherules shown in Figs. 55-56 have had such an origin, although it is possible that they were derived from the substance of a yolk-nucleus. During the early stages of yolk formation the cytoplasm at the outer boundary of the egg frequently appears vacuolated. This does not seem to be a constant phenomenon, however, and it may be due in part, at least, to the action of reagents.

In the egg of Bufo there are two generations of yolk-nuclei, both formed from or under the influence of the vitelline bodies, and both evidently concerned in the formation of yolk. The first yolk-nuclei that are formed appear simultaneously in different regions of the cytoplasm and they later move close to the germinal vesicle where they gradually fade away. One can readily trace every step in their development from the stage of that shown in Fig. 46 to that of Fig. 56. The yolk-nuclei belonging to the second generation are formed at the periphery of the egg and they are transformed directly into yolk spher-ules. The various stages in the development of these bodies can also easily be followed. With these facts in mind the following statement by Crampton (24) is of interest: "The accounts of the origin of the yolk-spheres from cytoplasmic elements at places removed from the nucleus, or from several centres, or in all parts of the egg at once, fail to take into consideration an earlier stage marked by the origin from the nucleus of a true yolk-matrix which subsequently disintegrates and spreads throughout the whole cell-body as in Molgula." It would seem as if enough cytological work had been done to make it clear that one cannot deduce general rules applicable to all eggs from the study of one particular egg, no matter how carefully the work may have been done.

According to my investigations the formation of the yolk in the egg of Bufo is closely associated with the vitelline bodies and also with the granular masses produced by them, the yolk-nuclei. I have elsewhere stated that the vitelline bodies are probably secretion products of the cytoplasm formed, possibly, under the influence of an enzyme given off by the nucleus. The

granular yolk-nuclei are undoubtedly composed of nutritive material which is subsequently aggregated into yolk spherules. In some instances the substance of the vitelline bodies seems to be transformed directly into yolk spherules; the intermediate stage, that of the formation of yolk-nuclei, being omitted. This would seem to indicate that the vitelline bodies are themselves but aggregations of nutritive material which is in a semi-fluid condition rather than in the form of granules. It may be that during the early stages in the development of the ova "yolk is present in the cytoplasm in the form of a diffused unstainable fluid," as Montgomery has suggested, and that this fluid is first collected into the rounded vitelline bodies and later changed into yolk spherules.

The part taken by the nucleus in the formation of yolk in the egg of Bufo is as yet obscured. I have never seen any nucleoli or any minute granules leave the nucleus which might have an influence on the formation of the yolk. If, as seems probable, the nucleus directs and controls the nutritive processes in the cell, then in the formation of yolk it must act either through a fluid substance which it gives out into the cell-body, or it must exert its influence directly on the deuto-plasmic substance of the cytoplasm. In many kinds of eggs, according to the investigations of Conklin, Crampton, Calkins, Foot and Floderus (31), the yolk is formed first around the nucleus and then produced progressively towards the periphery of the egg. In these cases it may be supposed that the cytoplasm surrounding the nucleus is directly stimulated by the nucleus to produce yolk. In amphibians and many other vertebrates the yolk first appears at the periphery of the egg. In these cases the nucleus has a less direct influence on the yolk formation, and this influence is probably exerted through the action of a fluid substance which passes by osmosis through the nuclear membrane into the cell-body. The investigations that have seemed to show that yolk is derived directly from nucleoli, or from chromatin, or from follicle cells, are all open to question, and until they have been confirmed by further research I shall be inclined to believe that yolk formation is

one of the anabolic processes in the cell which, although it is directly or indirectly controlled by the nucleus, does not depend upon the nucleus for its material substance.

ZoÖLOGICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA.
    March 14, 1908.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida. They have been reduced one-third.

Abbreviations used in lettering the figures:

Al., alimentary tract.
Ao., aorta.
C., centrosome.
C. V., cardinal vein.
E., endoderm.
G., germ-cells.
H., heart.
L., liver.
L. M., lateral plates of mesoderm.
M., mesoderm.
N., neural tube.
No., notochord.
Nu., nucleolus.
P., early prophase of mitosis.
T., Wolffian tubule.
V., vitelline body.

Fig. 18.—Cyst of secondary oögonia in the resting stage. × 1,334.

Fig. 19.—Cyst containing secondary oögonia in various stages of mitosis. × 1,334.

Fig. 20.—Young oöcyte with oval nucleus. × 1,334.

Fig. 21.—A slightly later stage than Fig. 20. The nucleus of the oöcyte has assumed a rounded form. × 1,334.

Fig. 22.—Early growth stage of the oöcyte. The nucleus contains a well defined, apparently continuous spireme. × 1,334.

Figs. 23-24.—Stages showing the gradual condensation of the nuclear substance previous to synizesis. × 1,334.

Fig. 25.—Synizesis stage. × 1,334.

Figs. 26-27.—Post-synizesis stages. Part of the chromatin has been evolved in the form of a continuous convoluted spireme: the nucleoli and the rest of the chromatin appear in the form of irregular masses lying against the nuclear wall or in the meshes of the spireme. × 1,334.

Figs. 28-29.—Stages showing the longitudinal splitting of the spireme. × 1,334.

Fig. 30.—Slightly later stage. The sister portions of the spireme have begun to separate. × 1,334.

Fig. 31.—Young oöcyte surrounded by its zona pellucida. In the nucleus the sister portions of the spireme are almost entirely separated. × 1,334.

Fig. 32.—Section of an oöcyte in which there is a complete separation of the sister portions of the spireme. × 1,334.

Figs. 33-34.—Nuclei of the young oöcytes showing the division of the spireme into double segments. × 1,334.

Fig. 35.—Section of the nucleus of a young oöcyte showing the beginning of the resolution of the amorphous masses shown in Figs. 26-34. × 1,334.

Fig. 36.—Section of a young oöcyte showing the differentiation of one of the amorphous masses into a meshwork of chromatin threads and rounded nucleoli. × 1,334.

Fig. 37.—Section of a cyst containing oöcytes in different stages of development. × 1,000.

Fig. 38.—Stage following that of Fig. 36, showing the relation of the nucleoli, the oxychromatin threads and the chromosomes. In the cytoplasm are numerous vitelline bodies. × 1,334.

Fig. 39.—Section of a young oöcyte. The chromosomes are scattered throughout the nucleus and they have assumed the feathery appearance which characterizes them throughout the rest of the growth period. The oxychromatin threads are entirely separated from the nucleoli and have become very granular. In the cytoplasm are numerous vitelline bodies of various sizes. × 1,334.
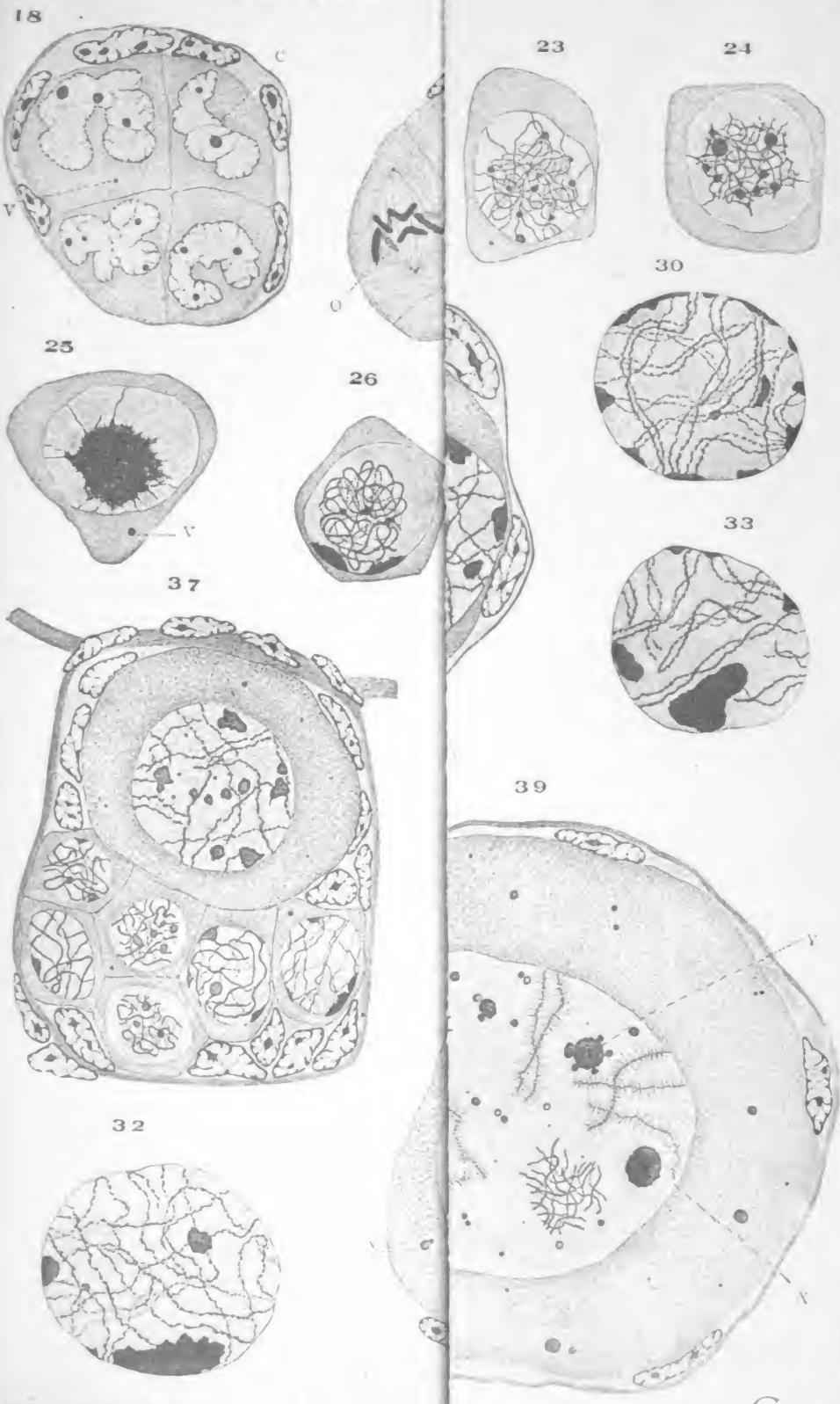
Figs. 40-41.—Sections of the nuclei in oöcytes of a young toad with a body length of 3.5 cm. A large nucleolar body, oxychromatin threads and feathery chromosomes are shown. × 1,000.

Fig. 42.—Section of the nucleus in the oöcyte of a toad with a body length of 3 cm. Some of the nucleoli stain faintly and are evidently in the process of dissolution. × 1,000.

Fig. 43.—Section of a nucleus in an oöcyte of a toad with a body length of 4 cm. showing the fragmentation of a large nucleolar body, scattered oxychromatin threads, and a pair of chromosomes. × 1,000.

Fig. 44.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. The yolk-nuclei are collected in a zone lying midway between the nucleus and the periphery of the egg. Diameter of the egg is 0.23 mm.; of the nucleus, 0.11 mm. × 1,000.

Fig. 45.—Drawn from an egg taken from the same ovary as that from which Fig. 44 was taken. Differentiation of the compound-nucleoli with the aid of safranin and gentian violet. × 1,000.

Fig. 46.—Part of a section of an egg taken from a toad with a body length of 5 cm. The yolk-nuclei are forming at the expense of the vitelline bodies. × 1,000.
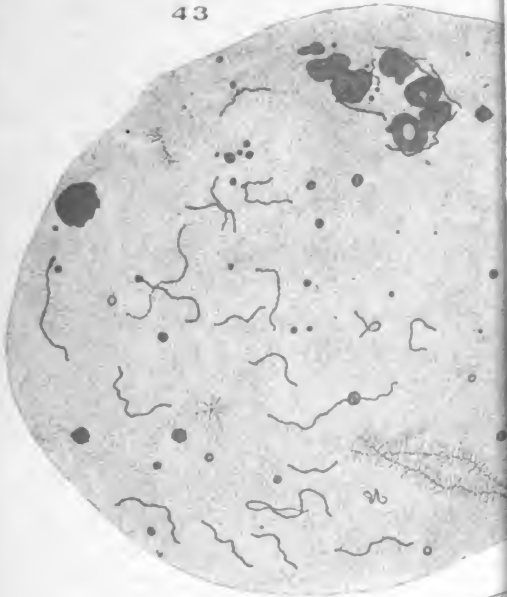
Fig. 47.—Division stages of a vitelline body. × 1,334.

Fig. 48.—Section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April. The plasmosomes are separated from the chromatin and most of them are massed at one side of the nucleus. Diameter of the nucleus, 0.2 mm. × 333.
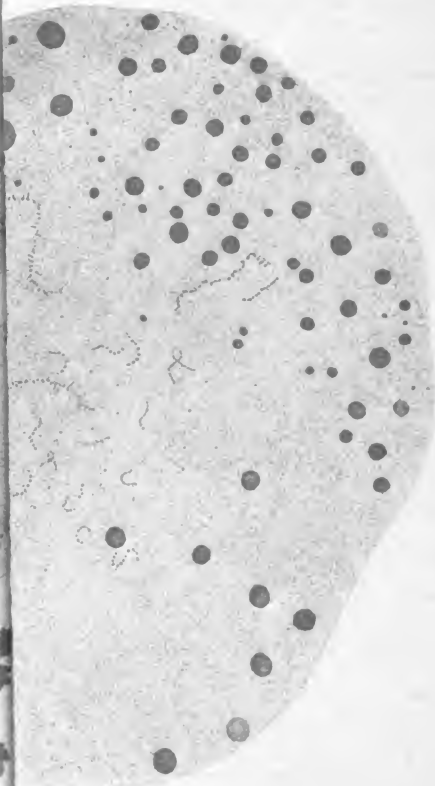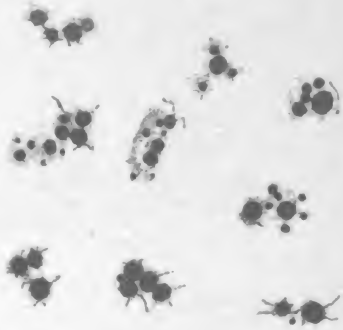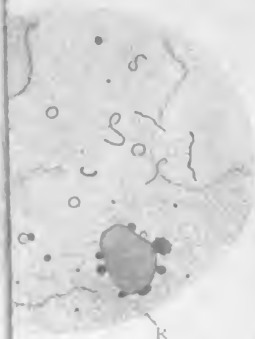
Fig. 49.—Section of the nucleus of an egg taken from an adult toad killed the latter part of April. The chromosomes occupy the centre of the nucleus, while the plasmosomes are very evenly distributed about the nuclear periphery. $\times$ 333.

Fig. 50.—Section of the nucleus of an egg taken from an adult toad killed early in May. The plasmosomes have migrated to the interior of the nucleus and they enclose the chromosomes. $\times$ 333.

Fig. 51.—Peculiar types of compound-nucleoli found in many of the nuclei during the later development of the oöcytes. $\times$ 1,334.

Fig. 52.—Stages showing the formation of yolk spherules from a vitelline body. $\times$ 1,334.

Fig. 53.—Yolk-nuclei and vitelline bodies in an egg taken from an adult toad killed the latter part of April. $\times$ 1,000.

Fig. 54.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. New yolk-nuclei are forming at the periphery of the egg, and the older ones closely surround the nucleus. $\times$ 1,000.

Fig. 55.—Formation of yolk spherules at the periphery of an egg taken from an adult toad killed in May. $\times$1,000.

Fig. 56.—Part of the section of an egg taken from an adult toad killed in May. At the periphery of the egg a layer of yolk spherules is forming at the expense of yolk-nuclei and vitelline bodies. The layer of yolk-nuclei around the nucleus is beginning to disappear. $\times$ 667.

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The Relation of Age to Fertility in the Rat

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

THE RELATION OF AGE TO FERTILITY IN THE RAT

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THREE FIGURES

Data have recently been obtained that show the complete breeding history of a considerable number of female rats. An analysis of these data with reference to the question of fertility and its relation to age seems desirable, since literature dealing with litter size in rodents (bibliography in 'The Rat,' Donaldson, '15) gives very little information on this point and fails to record the entire litter production of even one pair of animals.

The breeding records of seventy-six females that produced a total of 585 litters are used in this study. The majority of the females (50) were piebald or 'hooded' rats; the rest were either 'extracted' albinos (15) or 'extracted' grays (11). All three strains were derived from the F_2 generation of a cross between the wild Norway rat (Mus norvegicus) and the domesticated albino (Mus norvegicus albinus). Mention is made of the kind of rats used merely as a matter of reference. The conclusions drawn from the results are doubtless applicable also to other strains of rats.

All of the females lived to be at least sixteen months of age, the oldest dying at the age of twenty-three months. Under the conditions existing in the animal colony of The Wistar Institute a rat is usually in its prime at the age of seven or eight months, and after reaching twelve months of age it is classed as 'old.' Very few individuals live for more than twenty months, although all animals are kept under environmental conditions that are seemingly well suited to their needs. The relatively early death of the animals is due, in part at least, to the fact

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that seasonal changes in temperature in the region of Philadelphia render old animals very susceptible to pneumonia, the disease that invariably proves fatal to a rat of any age. In a more equable climate, like that of California, rats have been kept in good physical condition until they were four years old (Slonaker, '12).

In the rat the menopause usually appears at the age of fifteen to eighteen months (Donaldson, '15, p. 21). Data covering the litter production during the first sixteen months of life, therefore, may be assumed to show the actual fertility of the great majority of females. The word 'fertility' is here used as defined by Pearl and Surface ('09) to designate: "the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring." Fertility, according to this definition, is a much more comprehensive term than fecundity with which it is often confused. The latter, as suggested by Pearl and Surface should properly be used to signify only "the innate potential reproductive capacity of the individual organism as denoted by its ability to form and separate from the mature body germ cells."

Litter data for the three strains of rats are shown in table 1.

TABLE 1

Showing litter data for the three series of rats

	NUMBER OF BREEDING FEMALES	NUMBER OF LITTERS CAST	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF YOUNG	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
Piebald series...................	50	406	6.9	2798	1447	1351	107.1
Extracted albinos...............	15	88	6.2	548	279	269	103.7
Extracted grays................	11	91	6.7	609	310	299	103.6
	76	585	6.7	3955	2036	1919	106.1

As table 1 shows, the corresponding records for the three series are very similar. The differences in regard to litter size and to the relative proportion of the sexes that are found are well within the limits of the variation that is always to be ex-

pected when the number of records is comparatively small. For further analysis, therefore, the data for the three strains have been combined. The entire series of records, arranged according to the age of the mothers when the litters were cast, is given in table 2.

The 'mean age of the females,' as given in the first column of table 2, is the median point of a thirty day period in the life of

TABLE 2

Showing the number of litters in the combined series, together with the sex ratios and the coefficients of variation for litter size. Data arranged according to the age of the females when the litters were cast

MEAN AGE OF FEMALES IN DAYS	NUMBER OF LITTERS CAST	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALE	COEFFICIENTS OF VARIATIONS FOR LITTER SIZE
90	38	6.9	264	126	138	91.3	34.9
120	49	7.9	389	207	182	113.7	32.3
150	57	7.6	433	215	218	98.6	26.5
180	60	7.8	472	252	220	114.5	31.5
210	61	7.7	471	243	228	106.5	25.2
240	46	7.3	337	155	182	85.1	36.8
270	44	7.0	314	163	151	107.9	36.2
300	49	7.4	363	187	176	106.2	35.7
330	41	6.0	246	138	108	127.7	37.8
360	31	6.1	191	97	94	103.1	36.5
390	35	5.1	179	104	75	138.6	51.2
420	26	4.5	118	64	54	118.5	41.8
450	18	4.3	79	35	44	79.5	51.5
480	13	3.2	42	23	19	121.0	73.3
510	10	3.4	34	17	17	100.0	36.3
540	6	3.6	22	9	13	69.2	47.7
570	1	1.0	1	1			
	585	6.7	3,955	2,036	1,919	106.1	38.0

each animal, except in the two cases noted below. For example, the mean age '120 days' includes the records for all litters produced by females that were from 105 to 135 days of age when parturition occurred. The ninety day group is one exception to the above rule; it comprises litter records for a twenty day period only, as the youngest mother in the series was eighty-six days old when her first litter was cast. One female gave birth to a litter of one when she was 594 days old. For the

sake of uniformity this record is put under the mean age '570 days' which is thus extended to include a period of forty-four days.

The majority of female rats that are in good physical condition cast their first litters when they are about three months old. Thirty-eight of the seventy-six breeding females bore young before they were 105 days old; all of the remaining females,
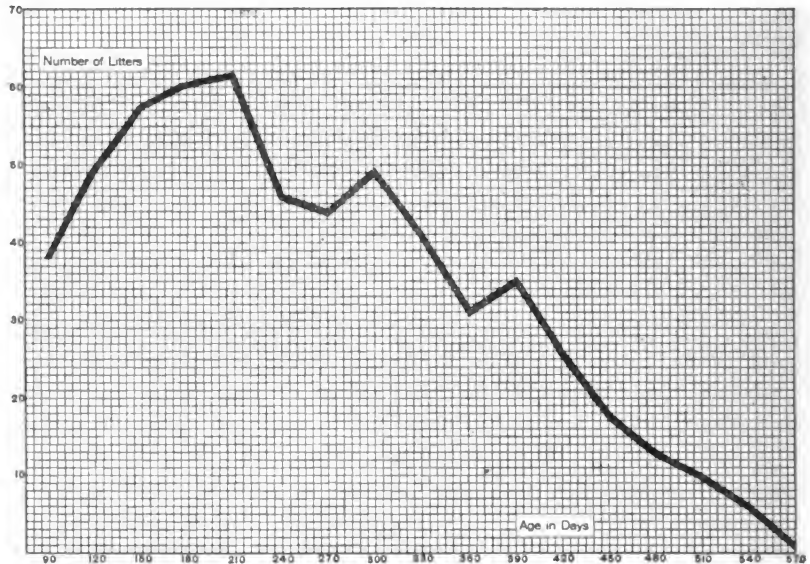

Fig. 1 Graph showing, for the entire series, the relation of the age of the mother to litter production (data in table 2).

with four exceptions, threw litters before they reached the age of 135 days. As table 2 shows, the number of litters cast increased with the age of the mothers until the females attained the mean age of 210 days. After the age of maximum fertility was passed the number of litters cast decreased rapidly, and only a small proportion of the females bore young after they had reached the age of fifteen months.

The graph in figure 1, constructed from the litter data in table 2, shows the relation of the age of the mother to litter production.

The graph in figure 1 starts relatively high and rises rapidly to its maximum which comes at the 210 day period. The decline of the graph is much more gradual than its rise, and not until near the 360 day period does the graph drop to the level at which it starts. From this point the fall is more rapid, and the graph reaches zero after the females have attained the mean age of 570 days. Fecundity in the rat, measured solely by the number of litters cast by the females at different age periods, is thus found to accord remarkably well with the law formulated by Marshall ('10): "The fecundity of the average individual woman may be described, therefore, as forming a wave which, starting from sterility, rises somewhat rapidly to its highest point, and then gradually falls again to sterility." There can be no doubt that animals, in general, tend to follow a similar law, as the litter records for various species collected by Marshall, by Pearl ('13) and others have already shown.

Judging from the data in table 2 a female rat reaches the height of her reproductive capacity when she is about seven months of age. This age represents also the median point in the animal's breeding career. That is, one-half of the total number of her offspring are produced by the time she has reached this age and one-half are produced afterwards.

When the females have reached the age of eighteen months their reproductive activity is usually at an end, as the data in table 2 indicate. Donaldson ('06) has shown that the first year of a rat's life is approximately equal to thirty years of human life. On this assumption a female rat that is eighteen months of age corresponds physiologically to a woman of forty-five. The menopause evidently takes place in these two forms at about the same period in the life span of the individual, but there is no corresponding likeness as regards the age of puberty or of maximum fertility; both of these processes take place in the rat at a relatively much earlier period.

The third column of table 2 shows the average size of the litters cast by the females at different age periods. The litters of very young females contained an average of 6.9 young per litter. This is a smaller average number of young than is found

for any group of litters until the females have past the zenith of their reproductive activity. Such a result was to be expected, since a number of investigations, for instance those of Minot ('91) on guinea pigs and of Hammond ('14) on rabbits and pigs, have shown that the number of offspring produced by young animals breeding for the first time is usually below the number that is considered normal for the species, and also that litter size tends to increase for a time with the age of the female. The largest litters in the series were those produced by females with a mean age of 120 days. Litter size remained close to the maximum until the females were eight months old when a slight diminution in the number of offspring was noticed. A further decrease to an average of only six young per litter was found in the litters thrown by females that were one year old. Each succeeding month added to the female's life seemed to lessen the number of her offspring to a marked extent, and after the females were fifteen months old the mean size of the litters cast was only about three .young per litter. Not infrequently the offspring of old females were born dead or soon died from neglect as the mothers seemed unable to suckle. them.

There is, as yet, no standard for litter size in 'extracted' strains of rats with which the present series of records can be compared. Miller ('11) and Crampe ('84) give 10.5 as the average number of young in a litter of wild gray rats; but Lantz ('10), on examining a large series of animals, found an average of only 8.1 embryos in pregnant gray females. According to Crampe the average litter of albino rats contains 6.3 young; data for over 1000 litters, collected by King and Stotsenburg, give the mean number of young in albino litters as 7.0. According to the above observations litters of gray rats contain a greater average number of young than do those of albino rats. The 585 litters used in the present investigation contained an average of only 6.7 young. This seems to indicate that litter size in 'extracted' strains of rats is less than that in either of the pure strains from which the animals were derived. It must not be forgotten, however, that the litter size for the pure strains, as given above, was not obtained from the complete breeding rec-

ords of a number of females but from a random collection of litters cast by females of unknown age. Litter size in various strains of rats cannot be properly compared until litter records for the several strains have been collected in a similar manner.

The relation between the age of the mother and litter size is shown by the graph in figure 2. The data used in constructing this graph are given in table 2.

The graph reaches its maximum when the females are practically at the beginning of their reproductive activity (i.e., at
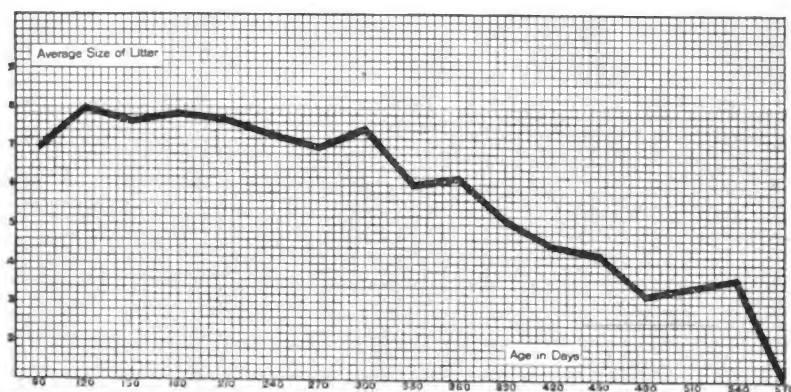

Fig. 2   Graph showing, for the entire series, the relation of the age of the mother to the average size of the litter (data in table 2).

120 days of age), and then declines very gradually approximating zero when the females are eighteen months old. Fertility in the rat, measured by the size of the litters cast, is thus found to be correlated with the age of the mother at the time that parturition occurs.

There is a possibility that the number of the pregnancy is a factor that influences the size of the litters cast. In order to analyze the data on this basis the records have been arranged according to the position of each litter in a litter series and are given in table 3.

When the data are arranged as in table 3 it is found that the second litter is the largest of the series. This result is in accord with the observations of Crampe ('84) and of King and Stotsen-

TABLE 3

*Showing the number, the average size of the litters and the sex ratios when the data
are arranged according to the position of each litter in a litter series*

POSITION OF THE LITTER IN A LITTER SERIES	TOTAL NUMBER OF LITTERS	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
1	76	7.2	553	290	263	110.2
2	76	7.7	591	292	299	97.6
3	76	6.9	531	276	255	108.2
4	74	7.3	543	270	273	98.9
5	66	7.0	463	235	228	103.0
6	56	6.8	384	197	187	105.3
7	43	6.9	300	157	143	109.7
8	36	5.8	210	118	92	128.2
9	30	5.0	151	89	62	143.5
10	21	4.8	100	57	43	132.5
11	14	4.8	67	31	36	86.1
12	9	3.8	35	15	20	75.0
13	7	3.5	25	9	16	56.2
14	1	2.0	2	0	2	
	585	6.7	3,955	2,036	1,919	106.1

burg ('15) on the albino rat. The number of the pregnancy,
up to five, does not seem to have a very marked effect on litter
size. The first five groups of litters have an average of 7.2
young per litter, which is above the average size of litters of
albino rats (7.0 young per litter) and considerably greater than
the mean size of all the litters in the present series (6.7 young per
litter). A slight decrease in size is noted in the sixth litter
group, and in the succeeding litters the number of young dimin-
ishes steadily. Only exceptionally vigorous females are able
to produce more than ten litters and these later litters rarely
contain more than one to three young.

As a rule female rats begin breeding when they are three
months old, and they will produce a litter each month for sev-
eral succeeding months if they are in good physical condition.
The second litter is cast, therefore, when the female is about
four months old and the fifth litter is born when the mother is
seven or eight months old. On referring to table 2 it is found
that litters born when the females are four months old have a

greater average size than litters cast at any other age period, and that females reach the climax of their reproductive activity at about seven months of age. In both tables there is a rapid decrease in the size of the litters towards the end of the series. As far as the question of litter size is concerned the two tables are in complete agreement. Such a litter series as that in table 3 is necessarily an age series, and it is very probable that it is the age of the female and not the number of the pregnancy that is a determining factor for litter size.

The size of a newborn litter of rats depends, primarily, on the number of ova shed at a given period of ovulation that are capable of fertilization. Litter size, however, is not always indicative of the actual fecundity of the female, since the off-spring born represent only that portion of the fertilized ova that were capable of normal development. Not infrequently the examination of a gravid female will show one or several fertilized ova in the uterus that are more or less atrophic and so incapable of developing into normal embryos (Huber, '15). Such ova are usually absorbed in situ, and only very rarely are monstrosities found among the normal newborn young. According to Hammond ('14), the lower fertility of young sows as compared with that of adult animals is due to the fact that not so many ova are shed at each period of ovulation. This explanation for the change in the fertility of swine is doubtless applicable also to a similar change in the fertility of rats and of other animals. Very probably the lessened fertility of old animals as compared with that of animals in their prime is due to the same cause. Whether abnormal ova are more frequent in old females than in young ones and so help to diminish the fertility in later life has not, as yet, been determined.

The last column of table 2 gives the coefficients of variation for the size of the litters cast by the females at different age periods. These coefficients show that size variation is considerably greater in the litters thrown by very young females than in the litters produced by females at the height of their reproductive activity when they are seven months of age. The latter

group of litters has the lowest coefficient (25.2) in the entire series.

As the number of litters cast after the females were a year old was relatively small, the coefficients for later litter groups can have little value. There seems, however, to be a very marked tendency for litters cast by older females to exhibit a greater range of variability in size than is shown by the litters of young females, the maximum variability appearing in the litters produced by females when they were about sixteen months old.

The entire series of litters gives 38.00 as the coefficient of variation for litter size. This coefficient is practically the same as that for litter size in the mouse, which is 37.5 according to the records collected by Weldon ('07), but it is 10 points less than the coefficient for the number of human offspring (Powys, '05). The coefficient of variation for fertility is very high in all mammals, apparently, being at least 25 per cent in the several cases where it has already been determined (Surface, '08).

Different females—even sisters from the same litter—show marked variations in the number and in the size of the litters they produce. Whether such differences depend upon the inheritance of various fertility factors, or whether they are due to environment or to individual peculiarities of the females themselves remains to be determined.

Table 4 shows the number of litters produced by the seventy-six females whose breeding records are used in the present study.

As shown in table 4, the range of variation in the number of litters produced by different females was from three to fourteen with an average of 7.7 litters per female. One of the two females that cast only three litters did not breed until she was six months old when she gave birth to a litter of seven. A second litter, with nine young, was born when the mother was eight months old, and a final litter, containing seven young, one month later. This female lived to be seventeen months old and she appeared to be in good physical condition until shortly before her death. The other female casting only three litters had a very similar breeding history. Some diseased

TABLE 4

Showing the litter production of 76 female rats

NUMBER OF BREEDING FEMALES	NUMBER OF LITTERS CAST
2	3
8	4
10	5
13	6
7	7
6	8
8	9
9	10
4	11
2	12
6	13
1	14
76	585

condition of the generative organs was doubtless responsible for the small number of litters produced by these two females, as investigations being carried on in the animal colony of The Wistar Institute by Dr. Stotsenburg show that sterility in a female rat is usually due to the formation of ovarian cysts or to degenerative changes in the uterus.

According to Crampe ('84), female albino rats, as a rule, do not produce more than four or five litters: records collected by Miller show that the wild gray rat has relatively more litters than the albino rat. The average of 7.7 litters per female, found in the present series of animals, is undoubtedly too high for the general run of females. Twenty-three of the seventy-six breeding females in this series had a total of five or six litters only, and it seems probable that this is about the average number of litters produced by female rats in general.

While six females had thirteen litters each, only one female gave birth to fourteen litters. This latter case is so unusual that it seems worthy of special note. The complete litter data are given in table 5.

This female, a piebald, gave birth to her first litter on February 7 when she was ninety-five days old. This litter was ex-

Showing the litter production of a female piebald rat, that was born November 4, 1913, and died June 14, 1915

LITTER SERIES	DATE OF BIRTH	NUMBER YOUNG	MALES	FEMALES
1	February 7, 1914..........................	11	5	6
2	March 11, 1914............................	13	5	8
3	April 3, 1914..............................	8	6	2
4	April 30, 1914.............................	9	5	4
5	May 23, 1914..............................	9	6	3
6	June 20, 1914.............................	10	2	8
7	July 14, 1914..............................	11	5	6
8	August 12, 1914...........................	6	2	4
9	September 10, 1914........................	10	6	4
10	October 15, 1914..........................	·10	9	1
11	November 23, 1914........................	4	3	1
12	January 28, 1915..........................	3	2	1
13	March 26, 1915............................	3	3	0
14	April 28, 1915.............................	2	0	2
		109	59	50

ceptionally large for the first litter of so young a female as it contained eleven young. The second litter, with thirteen young, was cast the following month. It is rather remarkable that both of these litters should be so much larger than normal, since, as a rule, a very large first litter is followed by a comparatively small one, unless at least two months intervene between the birth of the litters. The female cast two litters in April, and subsequently she gave birth to a litter each month until she was twelve months old. With one exception each of these litters was larger than the average litter of albino rats. A marked decline in fertility was noted after the female was a year old: the intervals between litters became longer and the size of the litters decreased. The fourteenth litter, which contained only two young, was cast when the female was about seventeen months old, and although the female lived to be nearly twenty-two months old she did not breed again. During this long period of reproductive activity a total of 109 young were born, 59 males and 50 females. The median point in this female's breeding career was the same as that for the entire group of

females, namely seven months, and she produced an average of 7.8 young in each litter.

An examination of the individual records for each of the remaining females in the series that gave birth to a very large number of litters i.e., from eleven to thirteen, shows that in every instance the first litter cast was large, containing from nine to eleven individuals. In those cases where females produced less than six litters the first litter cast, with one exception, never contained more than seven young. The number of records is so small that no definite conclusions can be drawn from them, but they seem to indicate that the size of the first litter cast is somewhat of an index of the fertility of that particular female: a large first litter indicating that the female, if she keeps in good physical condition, will produce more litters than the average run of females. Crampe states that the second of a rat's litters is always the 'best' and that this litter is indicative of the size of subsequent litters. This observation has been confirmed only in part by the present series of records: the second litter is the largest of the series, but the size of this litter is not as indicative of the later fertility of the female as is the size of the first litter cast.

Individual rats show as marked differences in the number of young produced at one birth as they do in regard to the total number of litters cast. Litters cast by some females are almost always relatively large. The female whose litter record is given in table 5, for example, cast but one litter in the first ten that contained less than seven young. Some females never have a litter that contains more than seven young, while others females cast a large and a small litter alternately.

The litter frequencies in the three series of rats are shown in table 6, the range in litter size being from one to sixteen.

In table 6, as in table 1, there are slight differences in the corresponding data for the three series of rats that may or may not prove to be significant when larger series of records are analyzed. Litters of eight young were most frequent in the piebalds and in the extracted grays, while six was the most common number of young in the litters of extracted albinos. The data for the

litter frequencies in the combined series is shown in the form of a frequency graph in figure 3.

The graph in figure 3 has two modes, one at the point of six and the other at the point of eight young per litter. The graph thus appears to be compound, and it is possible that one of the two modal points corresponds to the degree of fertility normal for the wild Norway rat and the other to the degree of fertility that characterizes the albino rat, since these are the two strains from which the animals used for this study were derived. As the material is probably heterozygous as regards the factors for litter size, it does not seem advisable to attempt any analysis of the curve. It is of interest in this connection to note that the graph for litter frequencies in swine, as given by Went-

TABLE 6

Showing litter frequencies in the three series

SIZE OF LITTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16
Piebalds...................	6	20	32	35	31	56	40	71	48	28	17	12	8	1	1
Extracted albinos.........		5	8	7	9	20	14	8	12	4	1				
Extracted grays..........		3	9	6	13	12	10	15	11	9	1	1	1		
	6	28	49	48	53	88	64	94	71	41	19	13	9	1	1

worth and Aubel ('16), has three modal points; one at four, a second at eight, and a third at twelve pigs per litter. The first mode corresponds to the degree of fertility in the wild hog, the third is close to that of the most fecund of the domestic breeds of swine, and the third probably represents a heterozygous condition.

Evidence regarding the relation of the age of the mother to the sex of her offspring is conflicting. Statistics collected by Bidder ('78) and by Punnett ('03) show that there is a great excess of boys among the children of very young mothers, the relative number of boys decreasing at subsequent births until the mother is thirty. Among children of old mothers (i.e., over forty) the sex ratio is again very high. In the horse Wilchens ('86) found a relation between the age of the dam and the sex of her offspring very similar to that existing, apparently,

in the human race. On the other hand, Schultze's ('03) investigations on mice indicate that the age of the mother has seemingly no influence whatever on the sex of her young.

According to the observations of King and Stotsenburg the normal sex ratio in the albino rat is about 107.5 males to 100 females. As there are no available data regarding the normal sex ratio in other strains of rats the sex ratio in the albino rat is here taken as the standard with which to compare the sex ratios found in the present series of animals.
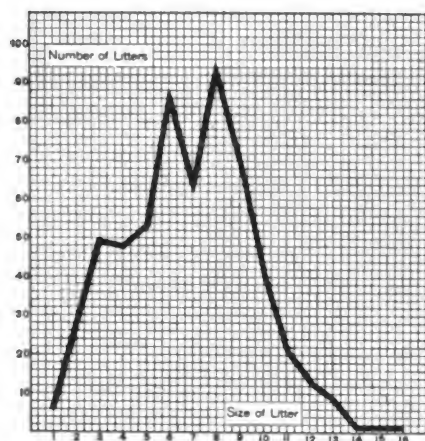
Fig. 3 Graph for the frequencies of litter size in the entire series (data in table 6).

Table 2 gives the sex ratios for the various litter groups when the data are arranged according to the mean age of the females at the time that the litters were cast. The sex ratios in litters belonging to closely related groups are so unlike that it would appear that there is no relation whatever between the age of the mother and the sex of her offspring. The sex ratio for the entire series of 3955 individuals is 106.1 males to 100 females. This shows that in the strains of rats used for this study the normal proportion of the sexes is about the same as that in the pure albino strain.

When the litter data are arranged according to the position of each litter in a litter series (table 3), the sex ratios obtained

for the individuals in successive groups of litters are not quite
as diverse as those for related litter groups as shown in table 2.
The sex ratio among the individuals belonging to the first lit-
ters of the series is higher than the standard, and in subsequent
litter groups, up to the fifth, there is seemingly a tendency for
the number of male offspring to decrease. A similar change in
the sex ratios from the first to the fourth litter was noted by
King and Stotsenburg in a series of litters cast by twenty-one
albino females. Beginning with the fifth litter the sex ratios
rise gradually until a maximum of 143.5 males to 100 females
is reached at the ninth litter of the series. For the eleventh
and subsequent litters, however, the sex ratios are much lower
than the standard. From the sex ratios as given in table 2
it would appear that among the individuals of a litter series
the sex ratio might be expected to start relatively high and then
fall steadily until about the fifth litter, rise again gradually to
a maximum at about the ninth or tenth litter and subsequently
drop to a low level which is maintained until the female reaches
the menopause.

The records under consideration are a special group selected
solely because they cover the complete breeding history of a
number of females that lived to an advanced age. Perhaps,
therefore, they cannot be used legitimately to give evidence
regarding the possible effects of the age of the mother on the
sex of her offspring. From the data as given the only conclu-
sion that can be drawn is that the age of the mother is not a
dominant factor in determining the sex of her young. If, as
Riddle ('16) maintains, sex is determined by the 'level of metab-
olism' in the fertilized egg, there is a possibility that the age
of the mother may indirectly influence sex through its effects
on the metabolic processes in the egg. Age has a profound
influence on every tissue in the body, and its effects on the
germ cells is a problem that must be attacked from a chemical
standpoint, since it can never be solved by sex statistics how-
ever extensive they may be.

SUMMARY

1. Litter data covering the entire breeding history of seventy-six female rats are given in the present paper. All of the females belonged to 'extracted' strains that were derived from the F_2 generation of a cross between the wild Norway rat and the domesticated albino.

2. The material used comprises the data for 585 litters containing 3955 individuals, 2036 males and 1919 females. The average number of young in each litter was 6.7.

3. Fertility in the rat, measured by the total number of litters cast, increases with the age of the female up to the time that the animal is seven months old. There is a sharp decline in fertility after the female is a year old and, except in rare instances, the menopause has appeared by the time that the female is eighteen months of age.

4. Female rats reach the height of their reproductive activity when they are about seven months of age. This age also represents the median point in the animal's breeding career.

5. The age of the mother is a factor in determining the size of the litter cast. Litters of very young mothers are relatively small, and later litters are large until the female reaches seven months of age. Litter size diminishes with the reduction in the number of litters cast, and litters of very old females rarely contain more than three young.

6. The second litter is the largest of the series, the third and fourth litters are usually a little larger than the first.

7. The serial number of the pregnancy, up to the fifth, does not seem to alter the size of the litter to any great extent. The sixth litter cast, however, is smaller than the preceding ones, and the number of offspring decreases rapidly as the position of the litter in the litter series advances. It is very probable that it is the age of the mother, not the number of the pregnancy, that influences the size of the litters.

8. Coefficients of variation for litter size show that the litters cast by very young females have a greater range of variation in size than have the litters cast by females at the height of

their reproductive activity. From this point the range of variation in litter size appears to increase as the female grows older, and to reach its maximum in the litters cast when the females are sixteen months old.

9. For the entire series of litters the coefficient of variation for litter size is 38.00.

10. The total number of litters produced by different females varied from three to sixteen, with an average of 7.7 litters per female.

11. The majority of female rats probably produce from five to six litters only.

12. The size of the first litter cast seems to be somewhat of an index of the fertility of the female. If the first litter is very large the female will probably cast more litters than the average run of females, provided she remains in good physical condition.

13. The range in litter size was from one to sixteen. Eight was the most frequent number of young in the litters of the piebalds and of the extracted grays, while six was the most common number for the litters of the extracted albinos.

14. The sex ratio for the 3955 individuals in the series was 106.1 males to 100 females. This sex ratio is very close to the normal sex ratio for the pure albino strain (107.5 males to 100 females).

15. The sex ratios obtained for the various litter groups (tables 1 and 2) do not indicate that the age of the mother is a dominant factor in determining the sex of her offspring. Old females, however, seem to produce relatively more females than male young.

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THE ANATOMICAL RECORD

CONTENTS

asonal variations in fertility and in
the sex ratio of mammals, with
special reference to the rat

by

Helen Dean King

With 9 figures in the text

SEASONAL VARIATIONS IN FERTILITY AND IN THE SEX RATIO OF MAMMALS, WITH SPECIAL REFERENCE TO THE RAT.

By

HELEN DEAN KING.

The Wistar Institute of Anatomy and Biology, Philadelphia.

With 9 figures in the text.

It is well known that reproductive activity in the majority of mammals, especially in those which have been in close association with man for a long period of time, occurs in cycles which can be readily modified by many different factors. THUS HEAPE ('01) has shown that not only climate, confinement and the food supply influence reproductive cycles in domesticated animals to a marked extent, but that habits of life, and the special nervous, vascular and secretory peculiarities of individuals play an important rôle in reproduction. To wild animals in their natural habitat the nutrition available for nursing mothers, and for the young as soon as they are able to care for themselves, is of paramount importance. As the food supply depends in great measure upon changes in temperature and in moisture, the seasons of the year must have considerable influence on the rhythm of generative activity.

Sexual functions are affected to some extent by different conditions in different species of animals. The sexual season, however, as WESTERMARCK ('21) has aptly stated, "is adapted to the requirements of each species. It is fundamentally governed by the law that the young shall be born at the time which is most favorable for their survival, and the influence of seasonal conditions upon sexual functions, and the length of the period of gestation, are subordinate to this law". At the present time many of our domesticated mammals breed throughout the year. It seems probable, however, that this is the effect of prolonged changes in environmental and in nutritive conditions brought about by captivity or by domestication, and that originally these animals had a primitive breeding season in the spring 'when winter is turning into summer' (ARISTOTLE).

Data that have been presented by various investigators, either for or against the assumption that there is a seasonal variation in fertility and in the sex ratio, have, for the most part, been taken from the stud books of various Breeder's Associations or from government statistics

of human births in different countries. Very few of them have been obtained from records of animals bred under controlled conditions which would eliminate environmental and nutritive factors that might influence the reproductive activity of the individuals. Human birth statistics, as has often been stated, are very unreliable in that the laws regarding the registration of births differ greatly in various countries, and records of illegitimate births and of the stillborn are commonly omitted. Records of Breeder's Associations are still more unsatisfactory, since in many cases only animals of value are registered and no account is taken of stillborn young or of those that die soon after birth. There are, therefore, many sources of error in these statistics which may lead to erroneous conclusions when the data are analyzed.

The conflicting results of investigators regarding the effect of seasonal changes on fertility and on the sex ratio leave this question still an open one. It seems worth while, therefore, to present new material dealing with this subject, and to compare the data with those others have used for a similar purpose.

Breeding experiments with the rat, which I have conducted for many years in the animal colony of The Wistar Institute of Anatomy and Biology, have furnished an extensive series of data which can be used to show whether, under fairly uniform conditions of environment and of nutrition, variations appear in litter production and in the sex ratio which can be ascribed to the changing seasons af the year. After all, it is not the question as to how sex is determined that is of especial interest to the animal breeder, though this is a matter that greatly interests the scientist. It is the number of viable offspring born and the sex distribution among them that most concern him. If breeding at one definite season of the year tends to increase the number of young born, or to give a preponderance of the sex desired, the breeder profits by this knowledge.

The data for the rat, given in this paper, comprise a total of 16,487 litters, containing 113,709 individuals, born in the colony during the past eighteen years. Breeding animals have been maintained under conditions of environment and of nutrition that have been as uniform as it was possible to keep them. There has been no change during all these years in the kind of cage used to house the rats, in the character of the bedding material used, nor in the routine of caring for the animals. The food given has, of necessity, varied somewhat at different times. Since 1918, however, the rats have had a cooked ration, differing each day of the week, and green food (such as lettuce, spinach, cabbage etc.) once or twice a week. The chief variation in the food supply has been that more greens have been given in summer than in winter. The temperature in the colony room has varied considerably at different seasons of the year. The room has always been heated by steam in winter, however, so that the temperature has rarely fallen below 40° F. In summer the temperature has not often been higher than 90° F.

Cages containing pregnant animals have been examined daily, except Sunday, and on the birth of a litter a record of the number of young and the sex of

the individuals has been taken. The stillborn young have been included in all cases. To exclude them would not only reduce the size of the litter, but probably change the sex ratio, since males tend to predominate among such individuals (KING, '21). As stillborn young are frequently eaten by adults, if left for a few hours in the cage, it is essential that all litters be examined as soon after birth as possible if the records are to have much statistical value. As parturition seems to occur more frequently in the morning than at any other time of the day, the great majority of litters have been obtained at or soon after birth. Errors of omission must inevitably have occurred in long continued breeding experiments of this kind, but they have been reduced to a minimum and, it is hoped, they have not seriously affected the results.

For all rats, except the inbred Albinos, the data given cover the entire reproductive life of the females used for breeding. Under conditions existing in my colony, the reproductive period in the female rat extends over a period of approximately fifteen months. Outbred albino and piebald females, if in good condition, were always kept until they were eighteen months old, and many that showed exceptional fertility were not discarded until they were two years old. Hybrid and gray females do not, as a rule, begin breeding at as early an age as do Albinos, and their reproductive period, therefore, extends to a later age. These females were retained until they were twenty months old in all cases where they were in physical condition to bear young; some that continued breeding were kept until they were over two years old. For a study of the factors that may tend to influence the sex ratio in the young it is important that the total litter production of all females be obtained when possible, since the age of the mother has a decided influence on the sex ratio in the young (KING, '16).

All of the rats used in this study belong to the same species, *Mus norvegicus*. The common form, the 'gray' or 'brown' rat (frequently called the Norway rat), is the original wild type from which the Albino and various colored rats have been derived through mutation. My colleague, Dr. H. H. DONALDSON, has recently made a suggestion which I have been pleased to follow throughout this paper, namely, that the word 'gray' should be used to designate the wild type, and that the term 'Norway' should be employed only in the general sense to cover all rats belonging to the species *Mus norvegicus*.

If seasonal changes in temperature or in the food supply directly or indirectly influence the sex ratio, they must act at or before the time of conception, since in mammals sex undoubtedly is irrevocably determined by the time that the ova are fertilized. It has seemed advisable therefore to arrange all series of data used in this paper not according to the time when birth took place, but according to the time of conception as nearly as this time could be determined from the data available. The length of the gestation period varies more or less in all mammals, and in arranging data in this way errors are bound to occur, since in very few cases is the exact time of conception known. In the Norway rat birth takes place, as a rule, 21,5—22 days after conception (LONG and EVANS, '22), although the gestation period can be greatly prolonged if the mother is suckling another litter (KING, '13). The time of conception for all rats has been assumed to have occurred 22 days prior to birth. As the gestation period is so short, conception and parturition frequently take place in the same month and usually

in the same season of th e year, as is the case in the mouse which has a gestation period of the same length as that in the rat. Where the gestation period is much longer, as in the pig, dog and man, conception and birth occur in different seasons of the year, thus offering a greater opportunity for many factors to act during gestation that may tend to influence the number of young born and the 'secondary' sex ratio as well.

1. Seasonal variations in litter production and litter size.

The word 'fertility', as used in this paper, denotes the reproductive capacity of females as shown by the number of litters or of offspring produced. There are many factors that influence fertility in mammals. As DARWIN has shown, captivity, changes in nutrition and hybridization all produce marked changes in the number of offspring born. To these may be added the age of the mother for, has DUNCAN ('96) has stated, "fecundity may be described as forming a wave which, starting from sterility, rises somewhat rapidly to its highest point, and then gradually falls again to sterility". In the rat, fertility in albino, piebald and 'extracted' females increases with the age of the mother up to the time that the females are seven to eight months old when the height of reproductive capacity is reached and the females have produced about half of their total number of offspring. There is then a gradual decline, both in the frequency and in the size of the litters cast, until the menopause. Maximum fertility in gray Norways kept in captivity comes when the females are about a year old.

The largest series of data for the rat given in this paper is that for inbred Albinos. These data cover the litter records for fifty generations, and were obtained during the years 1909—1925. All individuals recorded were born to females that were the offspring of sibs from the same litter. A detailed analysis of the findings in only the first twenty-five generations of these inbred rats has been published (KING, '18, a, b, c; '19). As this experiment was conducted, each female selected for breeding was mated but four times. The data given, therefore, do not cover the entire reproductive life of the females, as do those for other Norways. According to CRAMPE ('84), albino females, as a rule, do not cast more than four or five litters, though this is much below the average for several series of Albinos in my colony. The data given probably include the entire litter output of a great many of the females, however, and therefore are suitable for this study.

The data for inbred albinos, comprising 8,931 litters containing 65,724 individuals, are given in table 1. They will be discussed later.

Data for outbred Albinos were obtained from various series of these rats that were bred during the past ten years, either as controls for ex-

Table 1. Showing litter production and the sex ratio in inbred albino rats. Data arranged according to the month and season when conception occurred.

Month of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
January . .	815	5884	7,2	2958	2926	101,09 ± 1,86
February .	709	5236	7,4	2654	2582	102,79 ± 1,91
March . . .	858	6376	7,4	3170	3206	98,88 ± 1,66
April . . .	866	6588	7,6	3327	3261	102,02 ± 1,84
May	766	5737	7,5	2845	2892	98,37 ± 1,75
June . . .	734	5521	7,5	2742	2779	98,67 ± 1,78
July	764	5533	7,2	2752	2781	98,99 ± 1,78
August. . .	741	5422	7,3	2761	2661	103,76 ± 1,89
September .	752	5482	7,3	2769	2713	102,06 ± 1,85
October . .	585	4380	7,5	2146	2234	96,06 ± 1,96
November .	653	4683	7,2	2309	2374	97,26 ± 1,91
December .	688	4882	7,1	2458	2424	101,40 ± 1,96
	8931	65724	7,4	32891	32833	100,18 ± 0,53

Summary of data by seasons.

Season of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
Spring (Mar.—May)	2490	18701	7,5	9342	9359	99,82 ± 0,98
Summer (June—Aug.)	2239	16476	7,4	8255	8221	100,41 ± 1,05
Autumn (Sept.–Nov.)	1990	14545	7,3	7224	7321	98,69 ± 1,10
Winter (Dec.—Feb.)	2212	16002	7,2	8070	7932	101,74 ± 1,08

periments in progress or for a study of the effect of the age of the mother or her young. Breeding animals were taken from the large colony of 'stock' Albinos maintained at The Wistar Institute for research purposes. These data, comprising 3,276 litters containing 20,515 individuals, are shown in table 2.

The gray rat has not been used as extensively for experimental purposes as has the Albino, chiefly because it is more difficult to handle. Its rate of growth, body structure and the normal weight of various organs have been determined, however, and are available for comparison with similar data from other Norways (DONALDSON, '24; KING, '23). A large colony of these rats has been maintained at The Wistar Institute since 1918, and the animals have become so far domesticated that they are relatively very tame as compared to wild Grays. Data

Table 2.   Showing litter production and the sex ratio in outbred albino rats. Data arranged according to the month and season when conception occurred.

Month of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
January . .	245	1 529	6,2	770	759	$101,45 \pm 3,98$
February .	241	1 454	6,0	705	749	$94,12 \pm 3,32$
March . . .	259	1 602	6,2	810	792	$102,27 \pm 3,44$
April . . .	306	1 911	6,2	975	936	$104,17 \pm 3,21$
May. . . .	303	1 886	6,2	917	969	$94,63 \pm 2,94$
June . . .	294	1 849	6,3	952	897	$106,13 \pm 3,32$
July. . . .	311	1 940	6,2	979	961	$101,87 \pm 3,12$
August. . .	277	1 755	6,3	849	906	$93,71 \pm 3,02$
September .	303	1 934	6,4	926	1 008	$91,86 \pm 2,72$
October . .	261	1 648	6,3	842	806	$104,47 \pm 3,46$
November .	232	1 446	6,2	737	709	$103,95 \pm 3,67$
December .	244	1 561	6,4	794	767	$103,52 \pm 3,53$
	3 276	20 515	6,3	10 256	10 259	$99,98 \pm 0,94$

Summary of data by seasons.

Season of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
Spring (Mar.—May)	868	5 399	6,2	2 702	2 697	$100,19 \pm 1,86$
Summer (June—Aug.)	882	5 544	6,3	2 780	2 764	$100,58 \pm 1,81$
Autumn (Sept.—Nov.)	796	5 028	6,3	2 505	2 523	$99,29 \pm 1,89$
Winter (Dec.—Feb.)	730	4 544	6,2	2 269	2 275	$99,73 \pm 1,99$

for 2401 litters containing 14829 individuals comprised in twelve generations of Grays, born and reared in captivity, are given in table 3.

In table 4, data for the litter output in several generations of piebald rats that originated in the F_2 generation of a cross between wild gray males and stock albino females have been combined with data for a series of F_1 and F_2 hybrids between Grays and Albinos. This combination of data seemed justified, since both series showed the effect of hybridization on the sex ratio. Moreover, the data in each group were about equal in number and showed no great monthly or seasonal differences, either in litter production or in the sex ratio. This series of data comprises 1879 litters containing 12641 individuals.

A comparison of the data in tables 1 to 4 shows that maximum fertility of breeding females, as expressed by the number of litters

Table 3. Showing litter production and the sex ratio in gray rats. Data arranged according to the month and season when conception occurred.

Month of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
January . .	201	1 227	6,1	620	607	102,14 ± 3,93
February .	206	1 283	6,2	625	658	94,98 ± 3,69
March . . .	201	1 255	6,2	625	630	99,21 ± 3,77
April . . .	239	1 538	6,4	782	756	103,44 ± 3,55
May	236	1 463	6,2	751	712	105,48 ± 3,70
June . . .	208	1 247	6,0	620	627	98,88 ± 3,77
July	237	1 376	5,8	703	673	104,46 ± 3,79
August. . .	245	1 527	6,2	757	770	98,31 ± 3,38
September .	206	1 276	6,2	625	651	96,01 ± 3,62
October . .	136	877	6,3	443	434	102,07 ± 4,65
November .	115	707	6,1	336	371	90,57 ± 4,61
December .	171	1 053	6,2	497	556	89,39 ± 3,72
	2 401	14 829	6,2	7 384	7 445	99,18 ± 1,09

Summary of data by seasons.

Season of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
Spring (Mar.—May)	676	4 256	6,3	2 158	2 098	102,86 ± 2,12
Summer (June—Aug.)	690	4 140	6,0	2 080	2 070	100,48 ± 2,21
Autumn (Sept.—Nov.)	457	2 860	6,2	1 404	1 456	96,43 ± 2,41
Winter (Dec.—Feb.)	578	3 563	6,2	1 742	1 821	96,14 ± 2,17

cast, does not come in the same month of the year in all Norways even when the animals are kept under the same conditions of environment and of nutrition. In inbred Albinos breeding seemingly reaches its highest point in April, while in outbred Albinos this maximum is in July; August is the best month for conceptions in the Grays, and February for the hybrid and piebald group. October and November are the months when sexual activity is at its lowest point in all races of Norways studied.

The monthly variations in litter production, shown in tables 1 to 4, offers no support whatever to the view, advanced some years ago by KING and STOTSENBURG ('15), that there is a biannual cycle in fertility in the rat, with high points in the spring and in the autumn. This much larger series of data for various races of Norways indicates that there is

Table 4. Showing litter production and the sex ratio in hybrid and piebald rats. Data arranged according to the month and season when conception occurred.

Month of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
January . .	159	1 107	6,9	591	516	114,57 ± 4,64
February .	182	1 240	6,8	635	605	104,95 ± 4,01
March . . .	174	1 140	6,5	542	598	90,64 ± 3,63
April . . .	176	1 213	6,9	644	569	113,18 ± 4,38
May	181	1 230	6,8	652	578	112,80 ± 4,30
June . . .	179	1 215	6,8	630	585	107,69 ± 4,16
July	153	976	6,4	488	488	100,00 ± 4,32
August . .	159	1 097	6,9	576	521	110,56 ± 4,50
September .	146	959	6,6	474	485	97,73 ± 4,25
October . .	105	687	6,5	330	357	92,16 ± 4,74
November .	133	890	6,7	438	452	96,90 ± 4,38
December .	132	887	6,7	452	435	103,91 ± 4,70
	1 879	12 641	6,7	6 452	6 189	104,25 ± 1,25

Summary of data by seasons.

Season of conception	Numbers litters	Number individuals	Av. nov. young per litter	Males	Females	No. males to 100 females
Spring (Mar.—May)	531	3 583	6,8	1 838	1 745	105,33 ± 2,37
Summer (June—Aug.)	491	3 288	6,7	1 694	1 594	106,27 ± 2,49
Autumn (Sept.—Nov.)	384	2 536	6,6	1 242	1 294	95,98 ± 2,56
Winter (Dec.—Feb.)	473	3 234	6,8	1 678	1 556	107,84 ± 2,56

but one pronounced sexual cycle during the year, with its highest point in the early part of the year and its lowest point in the autumn. The drop in the number of litters from the month of maximum to that of minimum breeding represents a loss of from 24,1 per cent (outbred Albinos) to 53,0 per cent (Grays).

When the monthly data for litter production (tables 1—4) are combined in four groups representing the four seasons into which we, in this part of the United States, are wont to divide the year, it is found that the maximum and minimum periods of litter production occur in opposite seasons of the year in three of the four series of data; in Grays the maximum in summer is followed by the minimum in autumn. These differences, it must be borne in mind, occur in al aboratory mammal allowed to breed freely at any season of the year and maintained under uniform and favorable conditions of nutrition and of environment.

The data given in tables 1 to 4 have been combined in table 5. This table naturally shows the influence of the preponderance of data for the inbred Albinos, which comprise over half of the total of 16 487 litters. The conclusions to be drawn from this great number of data regarding seasonal variations in litter production, however, are doubtless those that would be drawn from an examination of the data in any one of the first four tables.

Table 5. Showing litter production and the sex ratio in all series of rats.
Summary of data in tables 1—4.

Month of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
January . .	1 420	9 747	6,86	4 939	4 808	102,72 ± 1,39
February .	1 338	9 213	6,88	4 619	4 594	100,56 ± 1,41
March . . .	1 492	10 373	6,95	5 147	5 226	98,49 ± 1,31
April . . .	1 587	11 250	7,09	5 728	5 522	103,73 ± 1,32
May	1 486	10 316	6,94	5 165	5 151	100,27 ± 1,33
June . . .	1 415	9 832	6,95	4 944	4 888	101,15 ± 1,36
July . . .	1 465	9 825	6,70	4 922	4 903	100,39 ± 1,37
August . .	1 422	9 801	6,89	4 943	4 858	101,75 ± 1,38
September .	1 407	9 651	6,86	4 794	4 857	98,70 ± 1,36
October . .	1 087	7 592	6,98	3 761	3 831	98,07 ± 1,51
November .	1 133	7 726	6,82	3 820	3 906	97,79 ± 1,49
December .	1 235	8 383	6,78	4 201	4 182	101,45 ± 1,49
	16 487	113 709	6,89	56 983	56 726	100,45 ± 0,40

Summary of data by seasons

Season of conception	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
Spring (Mar.— May)	4565	31 939	6,99	16 040	15 899	100,89 ± 0,76
Summer (June—Aug.)	4302	29 458	6,85	14 809	14 649	101,09 ± 0,83
Autumn (Sept.—Nov.)	3627	24 969	6,88	12 375	12 594	98,26 ± 0,84
Winter (Dec.—Feb.)	3993	27 343	6,85	13 759	13 584	101,29 ± 0,83

A 'conception curve' for the rat, based on data for litter production as shown in table 5, is given in chart 1.

The trend of the graph in chart 1 is upward during the early part of the year to its highest point in April. It then falls gradually, with relatively slight fluctuations, until September and subsequently drops

to its lowest point in October. After this the rise is gradual again to its spring maximum. This graph shows clearly the yearly cycle in fertility that is indicated in the separate data for each race of Norways (tables 1—4).

In table 5 spring conceptions comprise 27,69 per cent of the yearly total, while autumn conceptions form but 22,0 per cent. The difference of 938 between the litters of spring and those of autumn represent 5,67 per cent of the total litter output for the year and a drop of 20,55 per cent in litter production. Such a difference is undoubtedly statistically important, even though not accompanied by a probable error. The fact
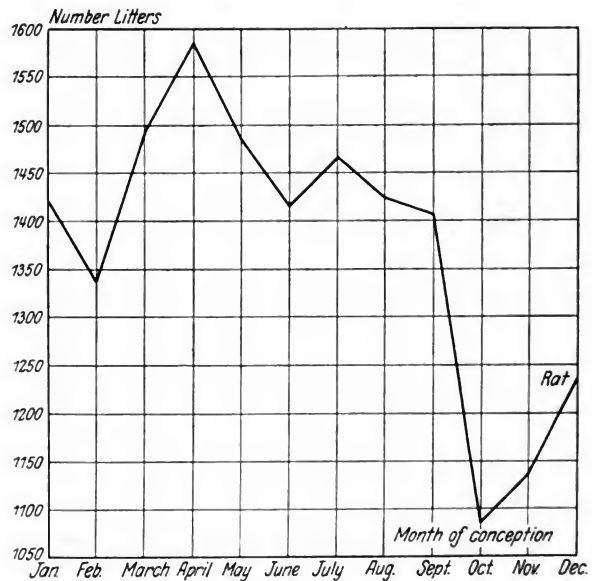
Chart 1. Graph showing monthly variations in litter production in the Norway rat (data in table 5).

that in each of the four series of data there is a very marked difference in litter production between the seasons of maximum and of minimum breeding adds considerably to the value of the difference shown in table 5, especially since the environmental conditions under which the animals were maintained eliminated many factors which are known to influence fertility in the rat.

From the results of the foregoing analysis of data it is evident that the Norway rat has retained traces of its primitive breeding season in the spring, although favorable conditions in captivity, which have brought abundant food and security from enemies, have tended to induce sexual activity at all seasons of the year.

The rat responds readily, and in many ways, to the changed con-

ditions of environment and of nutrition which captivity have brought, as is shown by the behavior of the gray Norways now under domestication in my colony. In no respect has this response been more evident than in their reproductive activity. In the first two or three generations, few females cast litters before they were seven or eight months of age, and many of them did not breed until they were a year old. During this time breeding took place chiefly in the spring or summer, very few litters were cast in autumn or winter. In later generations the females tended to breed at an earlier age and the breeding seasons was much less restricted. At present many gray rats of the fifteenth generation are breeding at four or five months of age and litters are being cast more uniformly throughout the year.

Seasonal differences in fertility are much more marked in gray Norways that have been in captivity but a short time than in other Norways. The data for these rats (table 3) indicates that 50,9 per cent more litters were conceived in the season of maximum than in that of minimum breeding. This difference is over twice as great as that in Albinos (21,1 per cent) — animals that have been under domestication for at least a hundred years. The gray rat, however, has been associated with man for a long period of time. This association has furnished it with a more abundant food supply, and has evidently influenced its reproductive activity, since wild Grays are known to breed throughout the year. Spring and summer, according to LANTZ ('10), being their chief breeding seasons.

The size of the litters cast furnishes another means of measuring fertility in the rat. In judging the value of variations found in litter size at different periods of the year caution is necessary, since not only do many factors act during gestation that produce a considerable amount of prenatal mortality, but there are individual peculiarities of the females that seem to play an important rôle in determining the number of young born in a given litter. Sisters from the same litter, kept under the same environmental conditions and mated to the same male, often show marked differences in the size, as well as in the frequency, of the litters that they cast. Some females never have more than five or six young at one birth; others cast a large and a small litter alternately. Exceptional females may cast large litters until near the close of the reproductive period (e. g. KING, '16; p. 280).

The average size of 1089 litters cast by young stock albino females in our colony during a period of three years was 7,0 (KING and STOTSENBURG, '15). Other determinations of litter size in the albino rat vary from 5,6 to 8,5 young per litter (CRAMPE, '84; CUÉNOT, '99; SLONAKER, and CARD, '23a; LONG and EVANS, '22). The mean, in general, for this race of Norways is around 7,0. Very few determinations have been

made for gray Norways, and all of these have been based on the findings in a very small number of litters. CRAMPE ('84) found an average of 10,4 young in fourteen litters of Grays; MILLER's ('11) data give 10,5 young as the average; while LANTZ ('10) states that the average number of fetuses in a large number of gravid females killed in India was 8,1. The only data for litter size in hybrid rats are those of CRAMPE, who obtained an average of 6,0 young in 398 litters. None of these data cover the reproductive life of the breeding females.

The data in tables 1 to 4 show that the average size for the total number of litters cast tends to vary somewhat in different races of Norways, although the range of variation is not great. The highest average (7,4), found in the inbred Albinos, is not due to inbreeding per se, but is undoubtedly to be attributed to the fact that only the first four litters of the breeding females were recorded and these litters tend to be the largest of the series cast. In the hybrids and piebalds the aver-
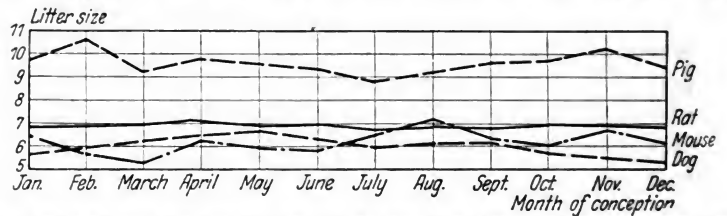

Chart 2. Graphs showing the monthly trend in average litter size in various mammals (data in tables 5, 8, 9; and in DIGHTON, '22).

age for all litters is 6,7 (table 4). Hybridization is known to raise the sex ratio in the rat (KING, '11), and it might be expected to increase the size of the litters also, since 'hybrid vigor' enhances the growth of the animals, renders them more resistant to the rat scourge 'pneumonia', and lengthens their span of life. The average size of the litters cast is practically the same in outbred Albinos and in Grays (6,2).

The range of variation in the monthly averages for litter size in any of the first four tables is extremely small. There seems to be a slight tendency for litters conceived in the spring months, especially in April, to be somewhat larger than those conceived in other months of the year, and for litter size to decrease towards the latter part of the year. In no case, however, is the average size of the litters more than 0,1 greater than the average for some other month of the year, nor is the lowest average more than 0,5 below the mean for the series. When the data in tables 1 to 4 are combined by seasons, variations in litter size are found to be insignificant.

Combined data for all rats (table 5) give 6,89 as the average size of the 16,487 litters. In this table all monthly averages are remarkably close to the mean for the entire series, none of them differ by more than

0,2. The highest average (7.09) is that for April conceptions; the lowest (6,70) for conceptions in July. The difference of 0,39 cannot be deemed significant. There is likewise no variation in the average size of the litters conceived in the different seasons of the year that is great enough to have statistical value.

A graph showing the monthly trend in litter size, constructed from the data in table 5, is shown in chart 2.

Since the graph for the rat, shown in chart 2, is practically a straight line, it indicates that there is no significant variation in the average size of the litters conceived in different months of the year.

Although the season of the year in which conception occurs has been shown to have considerable influence of the number on litters produced, it apparently has little, if any, influence on the average size of the litters. Litter size, as shown by these various series of data, is far more constant than any other litter characters that have been studied, such as the birth weight of the individuals and the sex ratio.

Since seasonal changes occur in reverse order in the two halves of the year, it may be of interest to compare litter production and litter size during the first six months of the year with those in the remainder of the year. In table 6 data for the various races of rats, as well as those for other mammals used for this study, have been grouped by semi-yearly periods.

A comparison of the various data for the rat, as given in table 6, shows that in each race more litters were conceived during the first half of the year than during the second half. From January until July a total of 989, or 12,76 per cent, more litters were conceived than from July until the end of the year. In all cases, except in the outbred Albinos, the average size of the litters conceived in the first period slightly exceeds that of the litters produced in the second period, but the excess for the combined data is only 0,12.

It appears, from this form of analysis of the data, that in spite of the uniformity in environmental and in nutritive conditions under which the breeding animals were maintained at all times, sexual activity in the Norway rat still has its maximum in that portion of the year which, under natural conditions, is most advantageous for the care and maintenance of the young. For wild animals having a short gestation period, conditions for rearing the young are steadily improving as the season changes from winter to summer. Food is becoming more plentiful, and the temperature more favorable. In the second part of the year, conditions constantly become less propitious for suckling mothers and for growing young. Food becomes more difficult to obtain and the animals suffer from inadequate protection against intense cold. The rigors of our northern winters are a severe tax on the strength and the

Table 6. Showing litter production and the sex ratio in variou|

	Animal	Number litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
		First Period (January—June)					
Rat	Inbred Albinos	4748	35 342	7,44	17 696	17 646	100,28 ± 0,7:
	Outbred Albinos	1648	10 231	6,20	5 129	5 102	100,53 ± 1,3
	Grays . .	1291	8 013	6,20	4 023	3 990	100,83 ± 1,5:
	Hybrids and Piebalds	1051	7 145	6,79	3 694	3 451	107,04 ± 1,7(
		8738	60 731	6,95	30 542	30 189	101,17 ± 0,5:
Mouse		174	1 024	5,88	514	510	100,78 ± 4,24
Pig		199	1 921	9,65	960	961	99,89 ± 3,07
Greyhound . .			18 791		10 054	8 737	115,07 ± 1,13

reserve force of many animals, and must necessarily influence their reproductive activity. It is only under favorable climatic conditions, or in captivity where shelter and sufficient food are provided, that they can breed and rear their young at all seasons of the year.

The Grays and the Albinos represent the extreme color varieties, as well as the wildest and the most domesticated, of the Norway rats. A comparison of the data for these rats, as given in tables 2 and 3, shows a remarkable similarity in the trend of seasonal variations in litter production, in the average size of the litters, and in the sex ratios. In both races more litters were conceived in summer than in any other season of the year, and there is a difference of only 0,1 in the average size of the litters produced. The difference of 0,80 between the sex ratios is negligible. A combination of the data for these two races may serve, perhaps, to give standards for litter size and for the sex ratio in the Norway rat which can be used for comparison with data from other series of Norways comprising the entire litter output of a number of breeding females. They will not be useful for comparison with data obtained by a 'random sampling' of the population in a rat colony where only young females are used for breeding, for reasons already stated. The combined data for these two races of Norways are given in table 7.

The average size of the 5,677 litters forming the entire series in table 7 is 6,2. This is a relatively low average, when compared with that usually give nas the 'norm' for the albino and for other Norway rats, but it

ammals data (tables 1—4, 7—9) combined in semi-yearly periods.

			Second Period (July—December)		
umber litters	Number individuals	Av. no. young per litter	Males	Females	No. males to 100 females
4183	30 382	7,26	15 195	15 187	100,05 ± 0,83
1628	10 284	6,31	5 127	5 157	99,42 ± 1,32
1110	6 816	6,14	3 361	3 455	97,28 ± 1,58
828	5 496	6,64	2 758	2 738	100,73 ± 1,82
7749	52 978	6,83	26 441	26 537	99,64 ± 0,58
129	838	6,57	454	394	115,23 ± 5,34
163	1 543	9,46	749	794	94,33 ± 3,24
	6 583		3 562	3 021	114,59 ± 1,91

more nearly represent the size of the litters one may expect to obtain when all litters cast by a series of females are recorded, and the still-born young included.

The sex ratio for the series (99,64 ♂; 100 ♀) is as near the 1 : 1 ratio as one could expect to obtain in a large series of data from any animal. The variation of this ratio from the norm as usually given for Norway rats, and a possible interpretation of its meaning, is reserved for the second section of this paper.

The extensive literature on the rat contains few references to seasonal variations in litter production and in the sex ratio. According to HANSON and SHOLES ('24) litter size in the albino rat is not affected by season. The average size of the litters, comprising 664 rats, varied from 5,97 in spring to 6,54 in winter. The difference of 0,57 ± 0,41, which is much greater than that found for any rats used in this study, is not deemed statistically important.

FELDMAN ('25), in a study of fertility in the yellow race of Nor-ways, found that the greatest number of litters (207) was cast in June (May conceptions), and that there was a rapid decline in fertility in the succeeding months until November when only 41 litters were born. His data show very pronounced monthly variations in the average size of the litters cast. Litters born before June showed a fluctuation of between 6,3 and 6,5 young per litter. The maximum (7,3) came for the litters born in June, and was followed by a steady decline to the low point (5,2) in October. After this time the size of the litters tended to in-

Table 7. Combination of data for litter production and for the sex ratio in outbred Albinos and Grays as given in tables 2—3.

Month of conception	Number litters	Number individuals	Aver. no. young per litter	Males	Females	No. males to 100 females
January . .	446	2 756	6,2	1 390	1 366	101,76 ± 2,61
February .	447	2 737	6,1	1 330	1 407	94,53 ± 2,44
March . . .	460	2 857	6,2	1 435	1 422	100,91 ± 2,54
April . . .	545	3 449	6,3	1 757	1 692	103,85 ± 2,38
May . . .	539	3 349	6,2	1 668	1 681	99,23 ± 2,29
June . . .	502	3 096	6,2	1 572	1 524	103,15 ± 2,49
July . . .	548	3 316	6,0	1 682	1 634	102,94 ± 2,41
August . .	522	3 282	6,3	1 606	1 676	95,82 ± 2,24
September .	509	3 210	6,3	1 551	1 659	93,49 ± 2,22
October . .	397	2 525	6,4	1 285	1 240	103,63 ± 2,77
November .	347	2 153	6,2	1 073	1 080	99,35 ± 2,89
December .	415	2 614	6,3	1 291	1 323	97,58 ± 2,57
	5 677	35 344	6,2	17 640	17 704	99,64 ± 0,71

Summary of data by seasons.

Season of conception	Number litters	Number individuals	Aver. no. young per litter	Males	Females	No. males to 100 females
Spring (Mar.—May)	1 544	9 655	6,3	4 860	4 795	101,36 ± 1,38
Summer (June—Aug.)	1 572	9 694	6,2	4 860	4 834	100,54 ± 1,37
Autumn (Sept.–Nov.)	1 253	7 888	6,3	3 909	3 979	98,22 ± 1,48
Winter (Dec.-Feb.)	1 308	8 107	6,2	4 011	4 096	97,92 ± 1,45

crease. It seems probable that some factor other than season must have induced these great variations in litter production and in litter size, although FELDMAN attributes them to the "devitalizing effect of high temperature and of high humidity in July and August which did not manifest itself until October".

Summer seems to favor the production of large litters in the guinea-pig. The chief factors involved, according to MINOT ('91), are complex nervous effects, not nutrition. WRIGHT ('22) offers no explanation for the increase other than to state that, "variations in environmental conditions have a marked influence on size of litter".

The only other data for any rodent that I have found suitable for analysis regarding the possible effect of seasonal changes on litter production and on litter size are those for the mouse, as given by PARKES

Table 8. Showing litter production and the sex ratio in the mouse. Data (PARKES, '26b) arranged according to the month and season when conception occurred.

Month of conception	Number litters	Number indivi-duals	Av. no. young per litter	Males	Females	No. males to 100 females	Month of birth
January .	20	129	6,45	64	65	96,91 ± 11,50	February
February .	12	68	5,66	37	31	119,35 ± 19,38	March
March . .	34	183	5,39	89	94	94,68 ± 9,44	April
April . . .	41	252	6,15	120	132	90,90 ± 7,72	May
May . . .	16	94	5,88	46	48	95,83 ± 13,26	June
June . . .	51	298	5,84	158	140	112,86 ± 8,83	July
July . . .	27	161	5,96	86	75	114,66 ± 12,10	August
August . .	40	290	7,26	147	143	102,79 ± 8,14	September
September.	16	102	6,38	52	50	104,00 ± 13,88	October
October. .	14	84	6,00	45	39	115,38 ± 16,99	November
November.	22	150	6,82	91	59	154,24 ± 17,22	December
December .	10	61	6,10	33	28	117,86 ± 20,42	January
	303	1872	6,18	968	904	107,08 ± 3,34	

Summary of data by seasons.

Season of conception	Number litters	Number indivi-duals	Av. no. young per litter	Males	Females	No. males to 100 females	Season of birth
Spring (Mar.-May)	91	529	5,81	255	274	93,06 ± 5,46	Apr.—June
Summer (June-Aug.)	118	749	6,35	391	358	109,22 ± 5,40	July—Sept.
Autumn (Sept.-Nov.)	52	336	6,46	188	148	127,03 ± 9,40	Oct.—Dec.
Winter (Dec.-Feb.)	42	258	6,14	134	124	108,06 ± 9,09	Jan.-March

('26b). The data in PARKES' tables 4 and 5, rearranged in accordance with the manner in which my own data for the rat are presented, are shown in table 8. Conception of the litters is assumed to have occurred in the month proceeding birth.

No statement is made by PARKES as to whether stillborn young were included in his data, nor is any reference made to the portion of the reproductive life of the females covered by the records. Presumably the data are for litters cast by relatively young females, since they came from a stock colony maintained for experimental work.

The mouse, as the rat, breeds at all months of the year if environmental conditions are favorable, as PARKES' data in table 8 show. Month-

ly variations in litter production are considerable, ranging from 51 in June to 10 in December. The combination of data by seasons shows that summer is the time of maximum fertility and winter the season of minimum fertility in the albino mouse, as in the albino rat (table 2).

As PARKES states, the monthly averages for litter size in his data for the mouse are not very coherent, although they show no very marked variations. The highest average (7,26) is that for litters conceived in August; the lowest for March conceptions. The difference between these averages (1,87) is considerable, but whether it is statistically important, considering the small number of litters involved, cannot be determined from the data as given. A graph for monthly averages in litter size for the mouse is shown in chart 2. The graph runs below that for the rat during its entire course except at its high point in August.

In the mouse, according to PARKES, fertility is higher in the second half of the year than in the first half. When his data are arranged by semi-yearly periods according to the time of conception, not of birth, this statement does not hold true for litter production. As shown in table 6, a total of 45, or 34,9 per cent, more litters were conceived in the first period of the year than in the second period, but the average size of the litters is greater in the second period.

The difference of 0,65 between the extreme values for litter size in the mouse when the data are arranged according to seasons (table 8) cannot be very important statistically, and therefore there is little reason to suppose that litter size in the mouse is influenced to any marked degree by the season of the year in which conception occurs.

MACHENS ('15) has given a series of data showing the influence of the month of conception on fertility and on the sex ratio of the pig. These data, which are for a white race of German swine, were taken from the herd books of the Züchtervereinigung für das veredelte Landschwein im Herzogtum Braunschweig. They are shown in table 9.

Although the breeding of pigs is often restricted to definite seasons of the year to suit the convenience of the owner, this is one of the domesticated mammals which breeds freely at any season of the year. The total of 362 litters comprised in MACHENS' data (table 9) are distributed fairly uniformly throughout the various months of the year. Breeding is at its maximum in April and at its lowest point in November, but it is only by the combination of data in seasonal periods that the yearly cycle in fertility is shown. Here the highest number of conceptions is found to occur in spring and the lowest number in the autumn, with summer and winter transitional periods between the extremes and containing practically the same number of conceptions.

Table 9 shows two months in the year (February and November) when the average size of the litters is considerably greater than that in

any of the other months. From this finding in his data MACHENS concluded that fertility in the pig appears to be higher in cold than in warm months. The excess in the average size of the litters conceived in winter is but 0,72 greater than the average for the summer, and therefore cannot be very important statistically.

Table 9. Showing litter production and the sex ratio in the pig. Data (MACHENS, '15) arranged according to the month and season when conception occurred.

Month of conception	No. of litters	No. of individuals	Av. no. young per litter	Males	Females	No. males to 100 females	Month of birth
January . .	35	343	9,80	173	170	101,76 ± 7,98	May
February . .	25	263	10,52	118	145	81,38 ± 6,80	June
March . . .	37	344	9,29	173	171	101,17 ± 7,35	July
April	38	369	9,71	189	180	105,00 ± 7,37	August
May	32	304	9,50	163	141	115,60 ± 8,96	September
June	32	298	9,31	144	154	93,51 ± 7,31	October
July	29	257	8,86	130	127	102,36 ± 8,61	November
August . . .	30	278	9,27	122	156	78,20 ± 6,37	December
September .	24	229	9,54	101	128	78,91 ± 7,08	January
October. . .	25	241	9,64	122	119	102,52 ± 8,91	February
November. .	23	236	10,26	126	110	114,54±10,07	March
December . .	32	302	9,44	148	154	96,10 ± 7,46	April
	362	3464	9,57	1709	1755	97,38 ± 2,22	

Summary of data by seasons

Season of conception	No. of litters	No. of individuals	Av. no. young per litter	Males	Females	No. males to 100 females	Season of birth
Spring (Mar.-May)	107	1017	9,50	525	492	106,71 ± 4,52	July-Sept.
Summer (June-Aug.)	91	833	9,15	396	437	90,62 ± 4,23	Oct.-Dec.
Autumn (Sept.-Nov.)	72	706	9,80	349	357	97,76 ± 5,07	Jan.-Mar.
Winter (Dec.-Feb.)	92	908	9,87	439	469	93,60 ± 4,19	Apr.-June

The monthly averages for litter size in the pig are shown graphically in chart 2. Aside from the two high points, one near the beginning the other near the end, this graph shows no marked variations in its course. The lowest point (July) is only slightly beneath the general level of the graph. Considering the probable omissions in the data, and the fact that prenatal mortality is common in this mammal, causing the loss of about 40 per cent of the ova shed (HAMMOND, '14), MACHENS'

data do not indicate that litter size in the pig depends in any way upon the season of conception.

When arranged in semi-yearly periods, MACHENS' data show that conceptions are more numerous and litter size greater in the first part of the year than in the second part (table 6).

From the monthly variations in 464 litters containing 3724 individuals, CARMICHAEL and RICE ('20) conclude that the time of year in which conception occurs has no noticeable effect on litter size in the pig. The variations found, however, are considerably greater than those shown by MACHENS' data.

PARKES' ('26a) extensive series of litter data for the pig, unfortunately, are not presented in a form in which they can be directly compared with those of MACHENS. They show that the average size of the litters farrowed is almost constant in the four quarters of the year, although over half of the births of the year take place in the spring.

The only other domesticated mammal for which data suitable for this study are available is the dog. HEAPE ('08) and DIGHTON ('22) each give a large series of birth data for the greyhound, taken from kennel records. These series of data, combined and rearranged, are shown in table 10.

HEAPE ('08) states that the best breeding times for the dog are in April and in September, but that for sporting reasons the greyhound is usually bred early in the year, as the age of the dogs is reckoned from January first of the year of birth regardless of the month in which it occurs. He also says that there are many omissions in kennel records, since all births are not recorded, especially if the litter is abnormal in size. The data in table 10, therefore, give no very reliable information regarding seasonal fertility in the dog. As they stand they indicate that breeding is at its maximum in spring, and that summer and autumn are seasons of relatively few conceptions. HEAPE's data for the collie show a much more uniform distribution of litters throughout the different months of the year. The maximum of conceptions in this breed seems to come in summer; the minimum in autumn.

DIGHTON ('22) has arranged his data to show the monthly variations in the average size of the litters. The highest average (6,65) comes in litters conceived in May; the lowest average (5,39) for litters conceived in December. The graph showing the monthly averages in litter size for this series (chart 2) indicates that in the dog, as in the other mammals cited, the size of the litter varies but slightly from the mean (5,9) at different periods of the year.

Civilization, with its complex customs, manners and social usages, has largely eliminated a definite breeding season in man. Such a breeding season must have existed, however, in early human ancestors. Man-

Table 10. Showing fertility and the sex ratio in the greyhound. Data (HEAPE, '08; DIGHTON, '22) arranged according to the month and season when conception occurred.

Month of conception	No. of individuals	Males	Females	No. males to 100 females	Month of birth
January . .	3313	1748	1565	111,69 ± 2,61	March
February . .	3782	2018	1764	114,39 ± 2,50	April
March . . .	4228	2272	1956	116,15 ± 2,40	May
April	3373	1812	1561	115,44 ± 2,69	June
May	2631	1418	1213	116,90 ± 3,22	July
June	1464	786	678	115,93 ± 4,08	August
July	698	375	323	116,09 ± 5,93	September
August . . .	409	216	193	111,92 ± 7,47	October
September .	279	152	127	119,68 ± 9,79	November
October . .	187	100	87	114,94 ± 11,45	December
November .	2562	1413	1449	122,98 ± 3,31	January
December. .	2448	1306	1142	114,36 ± 3,12	February
	25374	13616	11758	115,80 ± 0,98	

Summary of data by seasons.

Season of conception	No. of individuals	Males	Females	No. males to 100 females	Season of birth
Spring (Mar.-May)	10232	5502	4730	116,32 ± 1,55	May-July
Summer (June-Aug.)	2571	1377	1194	115,33 ± 3,06	Aug.-Oct.
Autumn (Sept.-Nov.)	3028	1665	1363	122,16 ± 2,99	Nov.-Jan.
Winter (Dec.-Feb.)	9543	5072	4471	113,44 ± 1,52	Feb.-April

like apes, as WESTERMARCK ('21) has shown, have a definite pairing time, and in many primitive people, such as certain North American Indians, tribes in Hindustan, Esquimaux and Australian aborigines, breeding appears to be restricted to a particular season, usually spring. Moreover, in many different races of mankind at the present time, there seems to be an annual increase in reproductive activity in the spring which is generally assumed to be a survival of the primitive mating time.

There is a wealth of statistical data regarding human births in various countries and for many different races of man. NICHOLS ('07) collected a great number of these statistics and pointed out their various sources of error. A number of investigators have analyzed birth data relative to seasonal variations in fertility and in the sex ratio. GOEHLERT

('88), from a study of birth statistics collected from nearly every country in Europe, concludes that the month when conceptions are at the maximum point depends chiefly upon the location of the country. The farther south the country, the earlier the appearance of the spring and, therefore, the earlier the manifestation of the greatest reproductive activity. BONNIER's ('23) data for Sweden show that the highest number of conceptions in this country comes regularly in March; the lowest number in November. Data for births in Canterbury (1854—1873), collected by RIGDEN ('76), indicate that May and September are the months of highest and lowest productiveness in England. In South America, where the seasons are the reverse of those in the northern hemisphere, the maximum number of conceptions comes in September, the minimum in May. In Cuba there are apparently two sharply defined breeding seasons each year; one in the beginning of winter. the other in March (HEAPE, '09). Other analyses of data bearing on this subject might be cited, but those given are sufficient, I think, to show
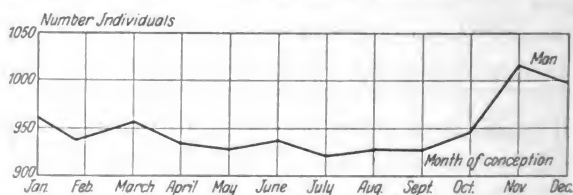

Chart 3. Graph showing monthly variations in conceptions in man (data in table 11).

that in different countries there is a well defined seasonal cycle in reproductive activity, with is highest point in spring and its lowest point in autumn.

Through the courtesy of Dr. C. B. DAVENPORT, Director of the Eugenics Record Office at Cold Spring Harbor, N. Y., I have obtained a unique and valuable series of data particularly suited for a study of this kind. These data were taken from the large series of family records filed permanently at the Eugenics Record Office. They show the sex composition of 1983 American families, each containing four or more children of the same mother. These data are as complete as one could hope to obtain any series of data for human births, since they were given by a member of each family concerned for the purpose of placing on record a complete history of family traits.

Table 11 shows these data, arranged according to the time when conception probably occurred, i. e., nine months previous to birth. This series of data has, in common with other series of data given, one unavoidable source of error. The gestation period in man varies considerably under different conditions; it may be extended to eleven months or shortened to seven or eight. To arrange data on the arbitrary assump-

Table 11. Showing the sex-composition of 1983 American families each containing four or more children. Data (from the archives of The Eugenics Record Office, Cold Spring Harbor, N. Y.) arranged according to the month and season when conception occurred.

Month of conception	Total of individuals	Males	Females	No. males to 100 females	Month of birth
January . .	960	506	454	111,45 ± 4,85	October
February . .	939	493	446	110,54 ± 4,87	November
March . . .	953	504	449	112,25 ± 4,91	December
April	931	484	447	108,28 ± 4,84	January
May	927	488	439	111,16 ± 4,93	February
June	936	495	441	112,25 ± 4,95	March
July	920	493	427	115,46 ± 5,15	April
August . . .	928	452	476	94,96 ± 4,19	May
September .	927	474	453	104,64 ± 4,63	June
October . .	945	505	440	114,77 ± 5,04	July
November .	1016	530	486	109,05 ± 4,61	August
December .	999	504	495	101,82 ± 4,34	September
	11381	5928	5453	108,71 ± 1,37	

Summary of data by seasons

Season of conception	Total no. individuals	Males	Females	No. males to 100 females	Season of birth
Spring (Mar.-May)	2811	1476	1335	110,56 ± 2,81	Dec.-Feb.
Summer (June-Aug.)	2784	1440	1344	107,14 ± 2,74	March-May
Autumn (Sept.-Nov.)	2888	1509	1379	109,43 ± 2,74	June-Aug.
Winter (Dec.-Feb.)	2898	1503	1395	107,74 ± 2,69	Sept.-Nov.

tion that the gestation period has been of the same length in all cases undoubtedly leads to many errors in the distribution of births over the various months of the year. These errors tend to balance each other, however, so they probably have but little effect on the data as a whole.

There is comparatively little difference in the number of conceptions for the different months shown in table 11. A 'conception curve', based on these data is shown in chart 3.

Throughout the early part of its course the graph in chart 3 runs at practically the same level. The dip to the low point comes for conceptions in July. Subsequently there is a gradual rise in the graph until the period of maximum reproductive activity is reached in November

6*

and December. There is no indication whatever in this graph of the spring rise in conceptions that is commonly shown by birth statistics for man, nor does a combination of the data by seasons show spring to be a period when the number of conceptions tends to increase.

As representative of American stock, in general, I have taken the data for human births in the registration area of the United States during the years 1915—1924 incl., as collected by the United States Census (Davis, '17—'26). Only living births are included in this series, since stillbirths were not recorded by the Census until very recently. These data, for over fourteen million births, are shown in table 12.

The conception data in table 12 indicate a biannual cycle in reproductive activity. There are two months of the year (June and November) when the number of conceptions is considerably greater than in the two months preceding or following. Corresponding low points are shown in the data for September and February.

For an 'Old World' stock with which to compare the data for American stock, I have selected the statistics for births in Prussia during the years 1872—1881 from the large number of such series of data available. These data, as given in Düsing's ('84) very comprehensive study, are divided so that one can compare legitimate with illegitimate births, and living with stillborn young. Living births only have been used for this study, since they are more comparable with those in table 12. The addition of all stillbirths (434 292) to living births does not appreciably change the relative number of conceptions during the different months of the year.

Düsing's data for living births in Prussia are shown in table 13.

Düsing's comment on the conception data in table 13 is that reproductive activity is strongest in June, weakest in September, and that the remaining months show regular transitions between these extremes except for the second rise in conceptions which occurs in December.

There is striking accord between the data in tables 12 and 13. Each series shows a well marked biannual cycle in reproductive activity; one conception maximum coming in June, the other at the end of the year (November—December). In each series there is one conception minimum in September and another in the early part of the year. The combination of data by seasons, however, eliminates one, though not the same, sexual cycle in each series.

The monthly trend in productiveness shown in the data from the Eugenics Record Office (table 11) does not accord with that in tables 12 and 13. In the former series only one sexual cycle is indicated (chart 3): in each of the latter series there is a biannual cycle. Which series of data more truly represents the yearly changes in reproductive activity in man generally cannot be determined. The data from the Eugenics

Table 12. Data for human births in the registration area of the United States during the years 1915—1924 incl. (U. S. Census reports).

Month of conception	Total no. individuals	Males	Females	No. males to 100 females	Month of birth
January . .	1 194,196	613 122	581 074	105,51 ± 0,14	October
February . .	1 112,333	570 814	541 519	105,41 ± 0,14	November
March . . .	1 141,006	586 464	554 542	105,75 ± 0,13	December
April. . . .	1 228,820	632 254	596 566	105,98 ± 0,13	January
May	1 167,317	598 508	568 809	105,21 ± 0,13	February
June	1 274,361	653 271	621 090	105,18 ± 0,12	March
July	1 195,256	614 219	581 037	105,71 ± 0,13	April
August . . .	1 212,580	624 772	587 808	106,29 ± 0,13	May
September .	1 174,205	604 853	569 352	106,23 ± 0,13	June
October . .	1 232,864	633 929	598 935	105,84 ± 0,13	July
November .	1 252,733	644 453	608 280	105,95 ± 0,13	August
December .	1 221,801	627 890	593 911	105,72 ± 0,13	September
	14,407,472	7,404,549	7,002,923	105,74 ± 0,035	

Summary of data by seasons.

Season of conception	Total no. of births	Males	Females	No. males to 100 females	Season of birth
Spring (Mar.-May)	3 537 143	1 817 226	1 719 917	105,66 ± 0,076	Dec.—Feb.
Summer (June-Aug.)	3 682 197	1 892 262	1 789 935	105,72 ± 0,074	Mar.—May
Autumn (Sept.-Nov.)	3 659 802	1 883 235	1 776 567	106,00 ± 0,074	June—Aug.
Winter (Dec.-Feb.)	3 528 330	1 811 826	1 716 504	105,55 ± 0,075	Sept.—Nov.

Record Office, though far more complete than those in the other two series, are not extensive enough to afford conclusive evidence that in man, as in lower mammals, there is but one yearly sexual cycle, although they indicate this strongly.

On combining the data for man by semi-yearly periods (table 14), it is found that the two American series accord in that more conceptions occur in the second half of the year than in the first half; the data for Prussia show a reverse relation.

The combination of data for 24 662 029 human births, as given in table 14, is influenced by the predominance of conceptions during the first part of the year in the Prussian data, therefore conceptions from January to July exceed those in the second period by 24 121. Con-

Table 13. Human births in Prussia (1872—1881 incl.). Data (DÜSING, '84) arranged according to the month and season when conception occurred

Month of conception	Total no. individuals	Males	Females	No. males to 100 females	Month of birth
January . .	873 436	448 398	425 038	$105,49 \pm 0,15$	October
February . .	843 202	432 562	410 640	$105,34 \pm 0,15$	November
March . . .	863 156	443 455	419 701	$105,66 \pm 0,15$	December
April	898 865	460 925	437 940	$105,25 \pm 0,15$	January
May	838 746	430 501	408 245	$105,45 \pm 0,16$	February
June	901 991	462 084	439 907	$105,04 \pm 0,15$	March
July	838 604	429 819	408 785	$105,14 \pm 0,16$	April
August . . .	832 315	426 609	405 706	$105,15 \pm 0,16$	May
September .	780 171	401 253	378 918	$105,89 \pm 0,16$	June
October. . .	819 460	421 593	397 867	$105,96 \pm 0,16$	July
November .	856 089	439 643	416 446	$105,57 \pm 0,15$	August
December. .	897 141	459 858	437 283	$105,16 \pm 0,15$	September
	10 243 176	5 256 700	4 986 476	$105,42 \pm 0,04$	

Sumary of data seasons.

Season of conception	Total no. births	Males	Females	No. males to 100 females	Season of birth
Spring Mar.-May)	2 600 767	1 334 881	1 265 886	$105,45 \pm 0,089$	Dec.-Feb.
Summer (June-Aug.)	2 572 910	1 318 512	1 254 398	$105,11 \pm 0,088$	Mar.-May
Autumn (Sept.-Nov.)	2 455 720	1 262 489	1 193 231	$105,80 \pm 0,091$	June-Aug.
Winter	2 613 779	1 340 818	1 272 961	$105,33 \pm 0,088$	Sept.-Nov.

sidering the very great number of individuals involved, the difference (1,9 per cent) in conceptions in the two periods is not a very significant one.

The foregoing analysis of various series of data indicate that in certain lower mammals, and also in man, there is a well defined seasonal variation in reproductive activity, as shown by the number of conceptions taking place at different times of the year. In order that a comparison may be made between the findings for the different species of mammals, the maximum and minimum periods of conception, by month and by season, are shown in table 15.

Table 15 brings out one point of some interest, namely, that the months of maximum and of minimum conceptions vary as much in different races of rats maintained under fairly uniform conditions of environment and of nutrition as in mammals that could not have been

bred under conditions as uniform or as favorable. All species, excepting man, have this in common: the period of the highest number of conceptions comes either in spring or in summer; that of the lowest number of conceptions in autumn or in winter.

The three series of data for man show, seemingly, hopeless discord, and for reasons already stated no conclusions from them seem justified. Whether this lack of agreement is due to statistical errors, to a difference in the racial stocks examined, or to differences in social customs and modes of living it is impossible to decide. That they are not caused by country or climate seems evident from the fact that the two American series of data show directly opposite seasons of maximum and minimum conceptions. A seasonal cycle in reproductive activity exists in man, without question, but whether this cycle is anual or biannual cannot be determined until a larger series of fairly complete data is available for analysis.

The data given for various domesticated mammals show clearly that seasonal variations in litter conception are not correlated with seasonal variations in litter size. Taking into consideration the incompleteness of some of the series of data, it appears that litter size does not tend to vary with the season in any of the mammals studied. Assuming that in mammals about the same number of ova are shed at each ovulation period, as Long and Evans ('22) state is the case in the rat, it would seen that the factors responsible for the heavy fetal mortality, which are probably largely physiological, tend to act uniformly throughout the year, consequently the average size of the litters remains practically unchanged.

It must be either through alterations in the food supply or through the rise and fall of temperature that the changing seasons influence reproductive activity in mammals.

Table 14. Data for human births combined in semi-yearly periods (tables 11—13).

Source of data	First period (January—June)				Second period (July—December)			
	No. individuals	Males	Females	No. males to 100 females	No. individuals	Males	Females	No. males to 100 females
Eugenics Record Office	5 646	2 970	2 676	110,98 ± 1,99	5 735	2 958	2 777	106,52 ± 1,89
U. S. Census 1915—1924	7 118 033	3 654 433	3 463 600	105,49 ± 0,053	7 289 439	3 750 116	3 539 323	105,96 ± 0,053
Düsing ('84)	5 219 396	2 677 925	2 541 471	105,37 ± 0,062	5 023 780	2 578 775	2 445 005	105,47 ± 0,064
	12,343 075	6 335 328	6 007 747	105,45 ± 0,040	12 318 954	6 331 849	5 987 105	105,76 ± 0,041

Table 15. Showing monthly and seasonal maxima and minima in conceptions and in the sex ratios of various mammals. Data in previous tables. E. R. O., Eugenics Record Office data; U. S. C., United States Census data.

	Month				Season			
	Number of conceptions		Sex ratio		Number of conceptions		Sex ratio	
	Maximum	Minimum	Highest	Lowest	Maximum	Minimum	Highest	Lowest
Inbred Albinos	April	Oct.	Aug.	Oct.	Spring	Autumn	Winter	Autumn
Outbred Albinos	July	Nov.	June	Sept.	Summer	Winter	Summer	Autumn
Grays	Aug.	Nov.	May	Dec.	Summer	Autumn	Spring	Winter
Piebalds and Hybrids	Feb.	Oct.	Jan.	Mar.	Spring	Autumn	Winter	Autumn
All rats	April	Oct.	April	Nov.	Spring	Autumn	Winter	Autumn
Mouse	June	Dec.	Nov.	April	Summer	Winter	Autumn	Spring
Pig	April	Nov.	May	Aug.	Spring	Autumn	Spring	Summer
Dog	March	Oct.	Nov.	Jan.	Spring	Summer	Autumn	Winter
Man E. R. O.	Nov.	July	July	Aug.	Winter	Summer	Spring	Summer
U. S. C.	June	Feb.	Aug.	June	Summer	Winter	Autumn	Winter
Düsing	June	Sept.	Oct.	June	Winter	Autumn	Autumn	Summer

Nutrition is the factor to which many investigators have ascribed the variations in the fertility of mammals occurring at different times of the year. This view assumes that the scarcity of food in winter lowers the vitality of animals and decreases sexual activity. With the increase of available food in the spring there is renewed vitality and hence a marked increase in reproduction. Nutrition, however, cannot play such an important rôle in the reproductive activity of laboratory mammals, as the rat and mouse, which are amply provided with food at all seasons of the year. In experiments in which the food given such animals has been inadequate in amount or not composed of constituents essential to maintain health, however, reproduction has been markedly affected in that sexual maturity has been delayed, litter production lessened and the size of the litters decreased (SLONAKER and CARD, 23'a, b; PARKES and DRUMMOND, '25).

It is through seasonal changes in temperature that the food supply of all mammals, including man, is chiefly regulated. HAYCROFT ('80), from his study of the effect of temperature on human births in Scot-

land, concludes that; "temperature is the main factor regulating the variation in the number of conceptions (and consequently of births) which occur during the year. It increases their number with its elevation, and this on an average of 0,5 per cent for an elevation of 1° F. — Increase of temperature is favorable to health and the increased capacity of conception in warm weather is only an indication of increased energy of the whole body in which this function (conception) shares." HAYCROFT seemingly overlooked one important fact. When temperature is raised beyond a certain point and maintained there for some time, it has a very devitalizing effect on man, as well as on other mammals, and then tends to check, not to stimulate, reproduction. Rats, which lack adequate means for heat regulation, suffer greatly from high temperature in summer. Reproductive activity is then greatly lessened and mortality, especially in older animals, is considerably increased.

Sexual activity, according to HEAPE ('09) always seems to be increased when there is a marked change in temperature, such as usually accompanies the turning of one season of the year into another. It is obviously not due to a higher or to a lower temperature alone, but is "essentially governed by the resultant of the various forces which are induced by a marked change of climate within certain limits".

Temperature is seemingly the one environmental factor that could have altered the reproductive rhythm in the various races of Norway rats maintained in my colony, since nutritive conditions were fairly constant. The temperature variations to which these rats have been subjected have had the same general trend, though they have not been as great, as those experienced by animals in their natural habitat. That changes of temperature likewise determine, to a greater or less extent, the periodic variations in reproductive activity in many animals living in the so-called 'temperate' regions of the world seems probable. Where the temperature changes are slight, as in tropical regions, humidity may be the factor that largely determines sexual activity.

Since the body temperature in higher mammals varies but little at different seasons of the year, temperature cannot affect reproduction directly by altering the condition of the uterine membranes and so influencing the union of ova and sperm, as has been suggested. Its influence, therefore, must be an indirect one.

A pronounced change of temperature, whether it is from the severe cold of winter to the more moderate temperature of spring, or from the heat of summer to the colder weather in late autumm, seems to 'tone up' the body generally, both in lower mammals and in man. This general increase in vigor is, primarily, an increase in body metabolism. The effect of this stimulus may persist for a longer or a shorter time, depending upon the age and physical condition of the individuals. It cannot

be maintained in any case, of course, unless nutrition is adequate. The reproductive organs are particularly sensitive to changes in environmental condition, as DARWIN has shown, and they might be expected to respond very quickly to changes in body metabolism. It is precisely at the time when temperature changes that favorably affect metabolic processes are most pronounced that reproductive activity is most manifest and the number of conceptions greatest. It is in early autumn, as a rule, when the high temperature and high humidity of summer have lowered body 'vigor' that generative activity is at its minimum.

The rise and fall of temperature at the changing of the seasons which stimulate metabolic activity thus indirectly seem to increase reproduction; extremes of temperature which lessen metabolism have the opposite effect. It is probably, therefore, that the changing seasons influence the breeding of mammals, through the effects of temperature on metabolic activity.

2. Seasonal variations in the sex ratio of mammals.

In the various tables in this paper the sex ratio is given as the number of males to each 100 females. There are advantages in presenting the sex ratio as the percentage of males in a given series of individuals, but the former method has been employed to accord with that used by most of the investigators whose data have been cited.

There seems to be some question at present as to how the probable error of the sex ratio should be calculated. The method that has frequently been used gives probable errors that are seemingly too low; a second method (YULE, '22) apparently errs in the opposite direction, since for small series of data or where the sex ratios show wide deviations from equality, the probable errors become so high that none of the ratios can be considered as statistically important. The number of individuals comprised in the various tables given in this paper is fairly large, therefore the second method of calculation the probable error of the sex ratio has been used.

Whether there is a seasonal variation in the sex ratio of mammals that can be correlated with seasonal variations in fertility is a question that has interested many investigators, and various series of data have been cited either in confirmation or in refutation of such a relation.

A former analysis of a series of data for the albino rat (KING and STOTSENBURG, '15) showed that the sex ratio was lowest in individuals born at the period of maximum fertility, and highest when reproduction was at its minimum. A similar inverse correlation between fertility and the sex ratio has been noted for mice (PARKES, 24b), and is indicated by HEAPE's ('08) data for greyhounds and collies as well as by various series of data for man (DÜSING, '84; GOEHLERT, '88; HEAPE, '09; COBB,

'14). On the other hand, data for the rat (HANSON and SHOLES, '24), for the pig (MACHENS, '15; PARKES, '26a), and for man (LEWIS and LEWIS, '06; BONNIER, '23) show no significant correlation between the sex ratio and the periodicity in reproductive activity that occurs at different times of the year.

It would seem to be a hopeless task to attempt to bring these diverse findings into any semblance of accord. Either the relation between the sex ratio and fertility differs greatly in various species of mammals, or the statistical data are at fault. The latter seems the more probable explanation.

A brief survey of the sex ratios in the various series of data cited will pave the way for a general consideration of this subject.

The two strains of inbred Albinos which furnished the data given in table 1 showed marked differences in their sex ratios for many generations (KING, '18c), yet when their data are combined the sex ratio in the entire series of 65,724 individuals is near equality (100,18 ♂ to 100 ♀). Variations from this mean are not great in any month of the year. Between the highest ratio (August) and the lowest ratio (October) there is a difference of 7,70 ± 2,53. Since this difference is slightly over three times its probable error, it may be considered as significant. Differences between the ratios for any other two months or between those for various seasons cannot de deemed as important. There is, seemingly, but one yearly cycle in the sex ratio of this series of Norways.

Monthly variations in the sex ratio for inbred Albinos are shown graphically in chart 4. The important changes in the sex ratio in these Norways occur in the latter port of the year, as the graph in chart 4 shows.

The sex ratio in the entire series of 20,515 outbred Albinos (table 2) agrees with that for the inbred Albinos in being close to equality (99,98 ♂ to 100 ♀); the monthly variations in the sex ratio, however, are much greater. The highest ratio is that for individuals conceived in June (106,13 ♂ to 100 ♀); the lowest for those conceived in September (91,86 ♂ to 100 ♀). The difference between these extreme ratios (14,27 ∓ 4,28) is well over three times its probable error and therefore may be considered as statistically important. In this series, also, seasonal differences between the ratios are unimportant.

The graph for the sex ratio in outbred Albino is shown by the unbroken line in chart 4. The course of this graph is very irregular with alternating high and low points. Only the highest and lowest points indicate statistically important differences in the ratio, and show the yearly trend.

In the grey Norways the sex ratio for the series of 14,829 individuals in again close to the 1 : 1 ratio. Monthly variations in the sex ratio,

shown graphically in chart 4, tend to be less than those in outbred Albinos. Between the highest ratio (May) and the lowest (December) the difference of 16,09 ± 5,24 is just over the borderland of 'statistical importance'. Seasonal differences in the ratio, although greater than those in the Albinos, are not important when judged by their probable errors.

The hybridization factor shows its effect in the hybrid and piebald group of Norways, and the sex ratio for this series of 12,641 individuals rises to 104,25 ♂ to 100 ♀. Monthly variations in the sex ratio are very great.
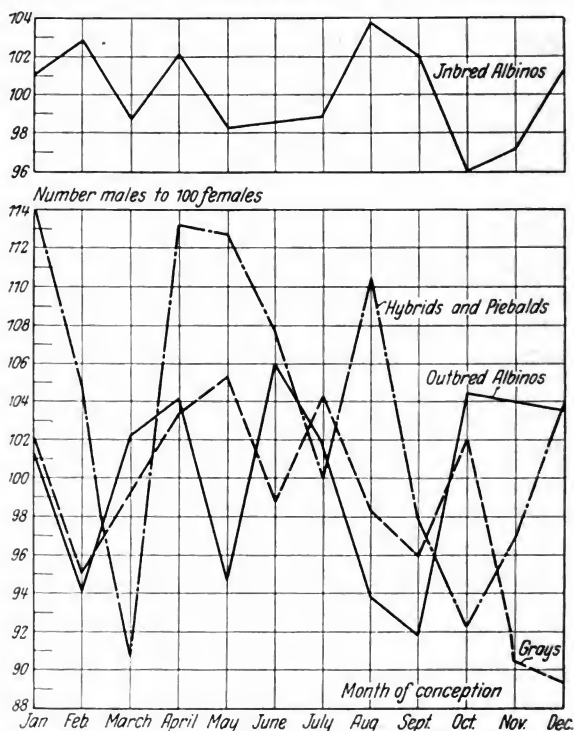
Chart 4. Graphs showing monthly variations in the sex ratio in different races of Norway rats (data in tables 1—4).

The graph in chart 4 for this series of Norways has two high points (January and April) which show significant differences from the low points in March and in October. This graph does not accord well with any of the other graphs for Norway rats shown in chart 4. Since hybridization in undoubtedly responsible for the high sex ratio in these Norways, it is probably also responsible for the great variations in the sex ratio found at different times of the year, as these rats were under the same environmental conditions as the other Norways.

The main conclusion that can be drawn from this survey of the trend of the sex ratios in various series of Norway rats is that there is a yearly, but not a strictly seasonal, cycle in the sex ratio. The highest point in the cycle comes in the spring or early summer; the lowest point in early autumn.

Graphs for seasonal variations in the sex ratio in each series of Norways are shown in chart 5. Comment on these graphs will be made later.

The combined data for all Norway rats, as given in table 5, shows a sex ratio of 100,45 ♂ to 100 ♀. Omitting the data for the hybrid and piebald group, since their sex ratio has undoubtedly been influenced by a definite factor (hybridization), there remains a total of 101 068 individuals in which the sex ratio is 99,88 ♂ to 100 ♀, or only 0,12 from equality. This ratio is as near the 1 : 1 ratio as the most ardent advocate of the chromosome theory of sex determination could ask.

Minor fluctuations in the monthly sex ratios tend to disappear in table 5, and the graph showing the general trend of these ratios (chart 8) is very different from any of those constructed for the separate races (chart 4). Only one difference between various ratios, that between the April high and the November low (5,93 ± 1,99), can be deemed important. This combined series of data, therefore, shows but one valid yearly cycle in the sex ratio.

Chart 5. Graphs showing seasonal variations in the sex ratio in different races of Norway rats (data in tables 1—4).

When the data for various races of Norway rats are arranged by semi-yearly periods (table 6), it is found that in every case the sex ratios are slightly higher for conceptions occuring in the first half of the year than for those in the second half. In no case, however, is the difference great enough to be considered as significant.

Until a more extensive series of data is available, the sex ratio in the combined data for outbred Albinos and for grey Norways (table 7), which is 99,64 ♂ to 100 ♀, will be considered as representative for Norway rats in general when the data given cover the reproductive life of the breeding females.

The equality in the proportion of the sexes found in all races of rats, except in the hybrid and piebald group, is an unexpected outcome of the analysis of these various series of data. The 'normal' sex ratio in the rat, usually given as about 107 ♂ to 100 ♀, is considerably higher than that found in the present series of data. I attribute the 1 : 1 ratio found
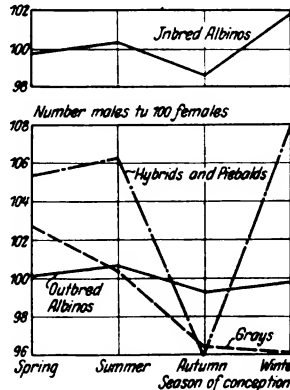
in these Norways chiefly to the fact that the great majority of the re-cords for all litters were taken at or shortly after parturition, and that these records cover practically the entire reproductive life of the breeding females. I do not think it due, to any extent, to the relatively large num-ber of individuals in the various series, to the environmental conditions under which the breeding animals were maintained, nor to the fact that breeding was continued through each year and not confined mainly to the 'collegiate' year of about nine months, which cuts out summer records and so materially changes the results.

The age of the mother seemingly influences the sex ratio in the rat quite as much as it influences her fertility in general. There are two periods in the life of the female rat (race immaterial) when the sex ratio in the litters that she casts tends to be very high, and there are two periods when this ratio is low. These two extremes apparently balance each other when the entire, unrestricted breeding history of the mother is shown. The usual method of discarding breeding females when they have passed the height of their reproductive activity give sa sex ratio for the portion of the reproductive period covered that is not represen-tative of the ratio for the whole of this period: it covers two periods when the sex ratio in the young tends to be high, and only one period when this ratio tends to the low. Whether there is a similar equality of the sexes in the young of other mammals when breeding records are fairly complete remains to be determined.

HANSON and SHOLES' ('24) records for the albino rat also show a seasonal cycle in the sex ratio, the proportion of males being highest among rats born in autumn and winter, and lowest for those born in the spring. The difference between these ratios, however, is not deemed statistically important. Since the data as presented by these investi-gators cannot be arranged according to the time of conception, the sex ratios given are not comparable with those for Albinos as given in this paper.

PARKES' ('26b) data for the mouse, when arranged according to the time of conception of the litters (table 8) gives sex ratios that vary greatly in different months of the year. The extraordinary high ratios in the late autumn are out of line with ratios for corresponding months found in the rat and in other mammals. The probable reason for these aberrant ratios, as well as for the drop in litter production in the latter part of the year, lies in the fact that the mice were removed to new quarters and were "subject to considerable disturbance during the following year". Since a great deviation from the mean in the ratio for any month is bound to influence the seasonal ratio containing that month, the combination of data by seasons gives ratios that are also very diverse. The difference of 29,81 $\pm$ 10,87 between the extreme ratios,

great though it is, fails to become significant because of the size of its error. The seasonal variations in the sex ratio for the mouse are shown by a graph in chart 6. The rapid and continued rise in the sex ratio from spring to autumn and its abrupt drop at this point are brought out clearly in chart 6. That there is one very pronounced seasonal cycle in the sex ratio is very evident.

SUMNER, McDANIEL and HUESTIS ('22) give a graph for monthly sex ratios in the deer-mouse, *Peromyscus*, based on data of conception. This graph is very different from that for any of the lower mammals shown in this paper in that it indicates a well marked biannual cycle in the sex ratio with high points in February and September and low ones in April and October. Since this graph was based on data obtained from matings that "were to a large extend controlled in accordance with the demands of the breeding experiments", it probably does not represent the normal seasonal trend of the sex ratio in this species.

The trend of the sex ratio in the pig (table 9) is very irregular, and shows a distinct biannual cycle. The only statistically important difference between the high and low ratios, however, is that between the May and August ratios (40,40 ± 10,99), so it must be assumed that there is only valid yearly cycle in the sex ratio of the pig. Seasonal changes in the sex ratio, which are shown in chart 6, are not important when judged by the error of their difference.

Chart 6. Graphs showing seasonal variations in the sex ratio in the mouse, pig and dog (data in tables 8—10).

Unfortunately, PARKES' (26a) large series of data for the pig are combined in four groups which do not correspond with the divisions of the year as adopted for this paper. The sex ratios given, PARKES states, show no signifiant variation, either in correlation with the birth rate or with any other factor.

The sex ratios obtained for the greyhound (table 10) are all very high, the mean (115,80 ♂ to 100 ♀) being much higher than that for any other mammal cited. Because of the omissions in the data due to restriction placed by breeders on reproduction, this series of ratios is of little value as representing the seasonal changes that probably
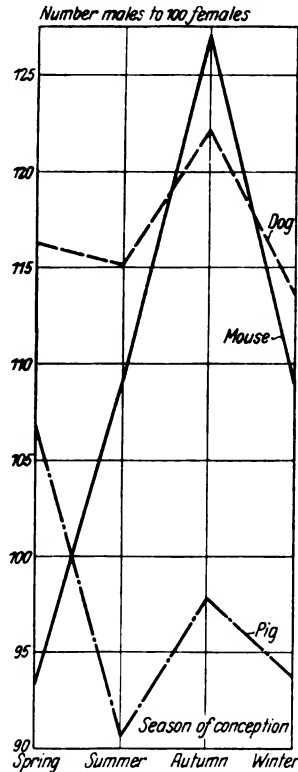
occur. Differences between the monthly and seasonal ratios are un-important.

With the exception of the greyhound, for which the data are inadequate, all of the lower mammals for which data are given show a yearly cycle in the sex ratio, though not a pronounced seasonal one when the differ-ences between the ratios are judged solely by their probable errors. The monthly variations in the sex ratio are very great in some cases, but these variations are not statistically important when judged by probable errors calculated by YULE's '(22) method. In every table given for these mammals one sex ratio appears that exceeds the lowest by a difference that is at least three times its probable error, and there-fore is presumably significant. These two ratios have been taken to represent the high and the low points in the yearly cycle. The months in which these extreme ratios appear vary in different mammals, but in general the highest ratio tends to appear in individuals conceived in a month of spring or summer; the lowest ratio is found more often in an autumn or winter month.

For the series of data for man obtained from the Eugenics Record Office the sex ratio is relatively high (108,71 ♂ to 100 ♀). This increase from the norm of 105,5 ♂ to 100 ♀ (NICHOLS, '07) may be due either to the completeness of the records, or to one of several factors that have been stated to influence the proportion of the sexes in the young at birth. Only families containing at least four children are included in this series (table 11). The ratio may be influenced, therefore by the fact that "sehr fruchtbare Ehen scheinen reicher an Knaben zu sein als minder fruchtbare und kinderarme Ehen" (GEISSLER, '89). The families furnishing these data belong mainly to the so-called 'upper classes' which live under more favorable nutritive and environmental conditions than do those of the laboring classes. In such families, PUNNETT ('03) has pointed out, the prenatal mortality which bears more heavily upon the males is lessened, since expectant mothers receive proper care and adequate nutrition.

Another fact that may have some bearing on the sex ratio in this series is that many of the marriages may have taken place between individuals of different racial stocks, since America is the 'melting pot' of races of mankind. Hybridization tends to increase the relative pro-portion of male offspring, as PEARL and PEARL ('08) have shown. A sex ratio of 108,3 ♂ to 100 ♀, which is remarkably close to that shown in table 11, has been found for another series of data from 3000 American families (NICHOLS, '05).

A graph based on the data for man in table 11 is shown in chart 7.

There are no important changes in the graph for man, shown in chart 7, during the early months of the year. The graph reaches its

highest point in July where the sex ratio is 115,46 ♂ to 100 ♀, and then falls abruptly to its lowest point in August. This drop represents a significant difference of 20,50 ± 6,64 between the extreme ratios. There
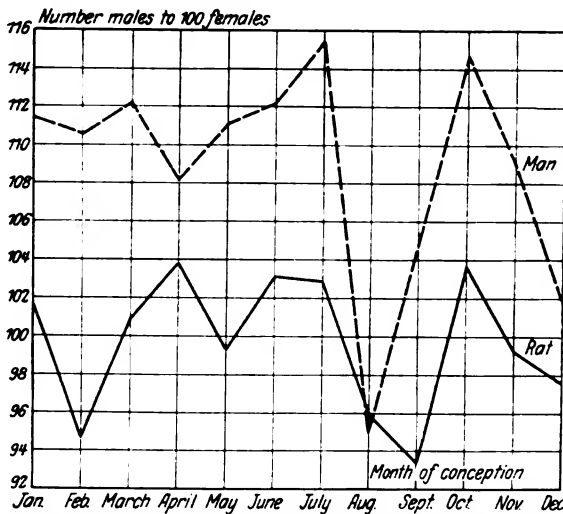

Chart 7. Graphs showing monthly variations in the sex ratio in man (data in table 11), and in the Norway rat (data in table 7).

is a subsequent rise to a second high point in October, which is 19,81 ± 6,55 above the August low, and a decline during the remainder of its course.
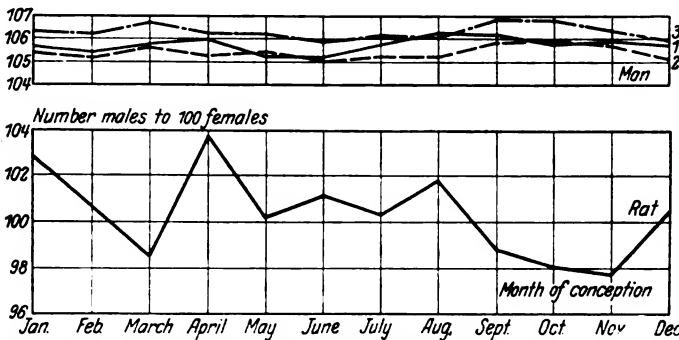

Chart 8. Graphs showing monthly variations in the sex ratio in man and in the Norway rat: graph 1 is based on the United States Census data (table 12); graph 2 was constructed from DÜSING's data for living births (table 13); graph 3 is based on DÜSING's data for all births. The graph for the rat is based on data in table 5.

Seasonal variations in the sex ratio for the Eugenics Record Office series of data for man are shown graphically in chart 9. They will be discussed later.

The very large series of data obtained from the United States Census reports (table 12), and from Düsing's records for births in Prussia (table 13) give sex ratios that seemingly vary little in the different months of the year. In such large series of data, however, slight variations are often significant. These ratios are shown by graphs 1 and 2 in chart 8.

A graph based on Düsing's data for all births is also included in chart 8 (graph 3). The addition of stillbirths to the data for living births raises the general level of the sex ratio from 105,42 to 106,28 ♂ to 100 ♀. It does not, however, appreciably change the trend of the ratios in the different months of the year, since graphs 2 and 3 run nearly parallel throughout their entire course.
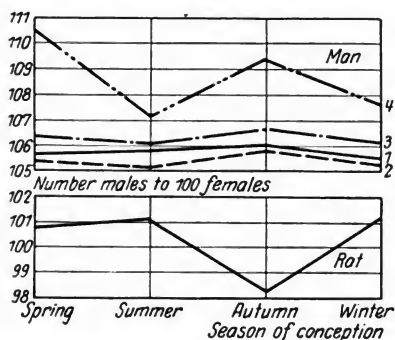
Chart 9. Graphs showing seasonal variations in the sex ratio in man and in the Norway rat. Lettering for graphs 1—3 as in chart 8. Graph 4 was constructed from the data obtained from the Eugenics Record Office (table 11); the graph for the rat on data in table 5.

In all three graphs for man shown in chart 8 there is evidence of a biannual cycle in the sex ratio. Graph 1, for the United States Census data, shows two high points; one for conceptions in April, the other for conceptions in August-September. Corresponding low points appear for conceptions in June and February. Differences between the high and low points are several times their probable errors and are significant without doubt.

Graph 2 and graph 3 in chart 8 (Düsing's data) have high points for March and October conceptions, and low points for conceptions in June and December. The differences between the extreme points are statistically important, though not by margins as great as those for the United States Census data.

The trend of seasonal variations in the sex ratio for all series for man are shown in chart 9.

The combination of data by season of conception gives sex ratios for the United States Census series that entirely eliminates the spring rise in the ratio that is indicated by the monthly data, since the course of graph 1 in chart 9 is upward from its beginning until it reaches its highest point in Autumn. Between the ratio in spring and that in autumn there is a difference of 0,34 ± 0,105, which may be deemed significant. Düsing's data show that the sex ratio for spring conceptions is slightly above that for summer conceptions, but not significantly so (0,34 ± 0,125). Graph 2 and graph 3 in chart 9, indicating the trend of the ratios in Düsing's data, run parallel and reach their highest point in autumn.

Graph 4 in chart 9, representing the ratio in the Eugenics Record Office series of data, indicates seasonal changes in the sex ratio quite unlike those of the monthly changes (chart 7). This graph has its highest point at its beginning (spring) and its lowest point in summer, the season which contains both the highest and the lowest monthly ratios. The subsequent rise in the graph (autumn) is not indicative of a biannual cyle in the sex ratio. In fact, none of the changes in the graph are important, since between the high ratio in spring and the low in summer there is a difference of only 3,42 ± 3,92. A yearly cycle in the sex ratio for this series is clearly indicated by the data in table 11 (chart 7), but seasonal variations in the ratio are not significant.

When the data for man are grouped by semi-yearly periods (table 14) it is found that the sex ratio for conceptions in the first part of the year is higher than that in the second part in the Eugenics Record Office series, though the difference between these ratios (4.46 ± 2.74) is not significant. On the other hand, ratios for the second part of the year are higher than those for the first part in both the United States Census and Dü- SING's series, the difference being important only in the former series (0,47 ± 0,07).

The summary given in table 15 will facilitate a comparison between the sex ratios for the various species of mammals and also form a basis on which to discuss the relation between fertility and the sex ratio.

In all Norway rats, except hybrids and piebalds, the sex ratio reaches its highest point in spring or summer and falls to its lowest point in autumn or winter, although the months in which the highest and lowest ratios appear vary in different races. The seasonal combination of data for all races brings the highest sex ratio in winter, but if the data for hybrids and piebalds are excluded because hybridization has probably influenced the ratios, the highest ratio comes in the summer months (100,45 ♂ to 100 ♀), and is only 0,12 above that for the spring.

Autumn is unquestionably the season when the sex ratio in the rat is at its lowest point. This is shown clearly by the graphs in chart 5. The difference between the high and the low points in these graphs are not important, if judged solely by their probable errors. The fact that all graphs show the same general trend should have some weight, I believe, when final conclusions are drawn.

There is no accord in the periods of highest and of lowest sex ratios for the mouse, pig and dog, as shown in table 15. Graphs for seasonal variations in the sex ratios of these mammals (chart 6) have one point of interest in common. Their course indicates that conceptions in autumn tend to produce a greater proportion of male offspring than do those at any other season of the year. These graphs show exactly the reverse trend in the sex ratio to that indicated by graphs for the rat in chart 5.

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This difference may mean either that various mammals react differently to seasonal conditions which affect the sex ratio or, which seems more probable, that the various series of data are not equally valid from a statistical standpoint.

The periods of high and of low sex ratios in the three series of data for man show little accord, although in all of them the lowest ratio comes in a summer month. Graphs for the seasonal variations in the ratios (chart 9) show a rise in the autumn which accords with the trend of the ratios in the mouse, pig and dog (chart 9), but not with those for the rat (chart 5).

The differences to be expected between the findings from a 'random sampling' of a given population and those from a fairly complete series of breeding records are well shown in the various series of data given in this paper. The very great diversity in these series makes it impossible to draw any conclusions from them as a whole. The most complete series of data are those for the various races of rats and for man (Eugenics Record Office). Considering, for the moment, only these data it is found that graphs showing the monthly trend in the sex ratios (chart 7) are much alike. Although the high and the low points in these graphs do not always come in the same month of the year, both graphs have the same general trend. The sharp decline in the graphs as summer wanes represents a decrease in the sex ratio that is statistically important in each case. The likeness between these graphs, both based on data more complete than any heretofore obtained for these species, is too great, it would seem, to be merely the result of chance. From these graphs it appears that in both the rat and in man the sex ratio tends to be high for conceptions in spring and summer, then to fall to a low point in early autumn. It rises again in October and subsequently is at a relatively low point until the late spring.

No exact relation between fertility and the sex ratio in mammals can be deduced from the summary of findings in table 15. Months of maximum and of minimum reproductive activity are at variance with those for the highest and the lowest sex ratio except in two of the Norway series. In four of the eleven series of records, however, the season of lowest productiveness in also that in which the sex ratio is at its lowest point; in not a single case is the reverse correlation found. That periods of high productiveness coincide with periods when the sex ratio is also high is not evident from table 15, but an examination of the various tables will show that the sex ratio is usually above the mean in months when conceptions are most numerous. There is little in these findings to indicate a reverse relation between fertility and the sex ratio in lower mammals as maintained by WILCKENS ('86, a, b), FRÖHLICH and GEORGS ('11), MACHENS ('15) and others.

In the three series of data for man the findings are discordant and out of line with those for lower mammals. The inverse relation between fertility and the sex ratio, brought out clearly in DÜSING's data, is not indicated in the United States Census data, since here one period of high productiveness (June) is associated with a low sex ratio while the second period of high productiveness (November) is not. Corresponding periods of minimum breeding show in one case (February) a low ratio and in the other (September) a high one (table 12). In the Eugenics Record Office data for man there is but one yearly cycle in both the number of conceptions and in the sex ratio. In the month when most conceptions occur (November) the sex ratio is above the mean for the year; in the month of the lowest number of conceptions (July) the sex ratio is at its highest point.

Since the average size of the litters in various races of rats, and also in other of the lower mammals, remains practically constant throughout all months of the year, it would seem that the pronounced monthly variations in the sex ratio must be due either to the action of factors that favor one sex at the time of conception, or to prenatal mortality that is selective in its action and tends to eliminate embryos of one sex more readily than it does those of the other sex at different times of the year.

The sexes should be conceived in equal numbers, according to the current chromosome theory of sex determination. To account for a high sex ratio at conception it has been assumed that male-producing spermatozoa, being smaller, are more active and have a greater rapidity of movement than have the female-producing spermatozoa, consequently they win the race to the ova more frequently. No proof of this assumption has ever been given.

At present there is no way of satisfactorily determining the sex ratio at conception (the primary sex ratio) in any mammal, although experimental work, like that of McDOWELL and LORD ('25a, b) on mice, offers promise of important developments in this field. The only method of approximately estimating the primary sex ratio is to obtain the fetuses dying in utero and to compare their sex ratio with the sex ratio at birth (the secondary sex ratio). A high prenatal sex ratio is indicative of a high primary sex ratio when the secondary ratio is near equality.

In pluriparous mammals prenatal mortality is difficult, if not impossible, to determine, since many of the dead embryos are absorbed in situ (HAMMOND, '14; HUBER, '15; LONG and PARKES, '24). In the rat prenatal mortality seems to eliminate about one fourth of the ova (LONG and EVANS '22). Stillborn young form about two per cent of the total number of offspring in this species and among them the sex ratio

is 129,3 ♂ to 100 ♀ (KING, '21). No determination has been made as yet of the sex ratio in fetuses dying before parturition. If prenatal mortality in this mammal eliminates as many males proportionally during gestation as it does in man the primary sex ratio must be close to PARKES' ('26a) estimation for the pig, mamely, 150 ♂ to 100 ♀.

From various determinations made for man, for whom alone among monoparous mammals data for abortions are available, it is evident that intrauterine mortality is very high. SCHULTZ ('21) estimates that out of every 100 fertilized ova only 78, or less, develop to term, the remainder being aborted. In all cases male fetuses die more frequently than female fetuses, and the earlier the age at which death occurs the greater the excess of males among them. AUERBACH ('12) found the sex ratio to be 229 ♂ to 100 ♀ for fetuses in the fourth month of gestation, and 116 ♂ to 100 ♀ for fetuses of the seventh month. High male mortality has been reported in man by NICHOLS ('07), RAUBER ('00), DAVIS ('17—'26) and others. From these findings it is evident that the primary sex ratio in man must be very high. AUERBACH estimates it at 125 ♂ to 100 ♀, although other investigators place it at from 108,74 ♂ to 100 ♀ (SCHULTZ, '12) to 111,0 ♂ to 100 ♀ (LENHOSSÉK, '03).

A variation in the number of aborted and of stillborn young in different seasons of the year might be expected to have an effect on seasonal variations in the sex ratio. DÜSING says that, "Bei den Kindern, welche im Anfang des Jahres erzeugt und im Herbst geboren werden, zeigen sich die wenigsten, dagegen bei denen, welche im Frühjahr gezeugt und im Winter geboren werden, die meisten Totgeburten." The addition of the stillbirths to the living young in DÜSING's series of data raises the ratio for spring conceptions 0,92 ± 0,12, and those for winter 0,84 ± 0,12. As the sex ratios are very high for stillbirths in all months of the year no important change in the trend of the ratios is produced by their inclusion.

Although the data for births in Sweden do not show any direct correlation between the sex ratio and the number of stillbirths, BONNIER ('23) ascribes the seasonal variations in the sex ratio, which he deems unimportant, to seasonal mortality. He assumes that through abortions and stillbirths there is a continuous equalization of the sex ratio. The rise in prenatal mortality at one season is supposed to have its disturbing effects distributed throughout the next eight months so that variations in prenatal mortality tend to balance each other as far as the ratio at birth is concerned.

Whether the variations in the sex ratio in man that occur during the year are due to changes in the number of stillbirths and abortions at different seasons or whether the primary sex ratio changes at different seasons is still to be determined. Evidence brought forward by

DÜSING and BONNIER seems to me to indicate that the yearly trend in the secondary sex ratio is not markedly affected by prenatal and birth mortality, since the variation in such deaths at different times of the year is not great and the mortality is invariably greater among male than among female fetuses.

In a former study of birth mortality in the albino rat (KING, '21) it was found that stillbirths were fairly evenly distributed throughout the year. The percentage of stillbirths in the total litter production, however, showed considerable seasonal variation, being nearly twice as great in autumn (3,04 per cent) as in summer (1,78). Unfortunately seasonal variations in the sex ratio among these stillborn young were not determined; but it could not have been great enough, considering the number of individuals involved, to influence the monthly secondary sex ratio. In the rat prenatal mortality seem to act fairly uniformly throughout the various months and seasons of the year, since the average size of the litters is not affected. Presumably it also acts to eliminate a greater number of male than of female fetuses, judging from conditions in man and from the fact that the sex ratio is very high in the stillborn. I am inclined to the opinion, therefore, that significant variations in the secondary sex ratio in the rat must be due to changes in the primary sex ratio, as PARKES ('24 b) concludes is the case in mice.

Arguments have been advanced in the first part of this paper in favor of the view that seasonal changes in temperature influence reproductive activity through their effect on body metabolism. From the findings regarding the relation between litter production and the sex ratio in various races of Norway rats it would appear that temperature also, in the same way, has an effect on the primary sex ratio in this species.

As a possible explanation of the way in which seasonal variations in the primary sex ratio might be produced, PARKES ('24b) states: "In animals with a marked breeding season, the spermatozoa must towards the end be matured in comparatively unfavorable conditions, and such conditions very likely have a greater lethal effect on the apparently less hardy X-spermatozoa than on the apparently more hardy Y-spermatozoa."

What the unfavorable conditions are that may affect the spermatozoa at the end of the breeding season, PARKES does not state. Assuming that they are environmental, it might be supposed that lower body metabolism induced by unfavorable temperature and inadequate food is the 'lethal' factor tending to reduce the number of male-producing spermatozoa and so to decrease the sex ratio at conception. This change would occur in autumn when reproductive activity is low. On the other hand, increased metabolic activity induced by the favorable rise

in temperature and more adequate nutrition in spring would tend to conserve the male-producing spermatozoa, and, assuming that they are the more active of the two kinds, this activity would give them enough advantage to insure a high primary sex ratio at this season of the year.

There is another way in which it seems possible to account for a periodic variation in the primary sex ratio of mammals. The chromosome theory of sex determination leaves the decision as to whether a given ovum shall develop into a male or into a female to chance, or places it upon the assumed greater abundance or greater activity of one kind of spermatozoa. It may be valid to assume that favorable or unfavorable conditions that alter body metabolism can change the relative proportion of the two kinds of spermatozoa, but I do not see why this assumption always implies that the ova are immune to such changes. There is considerable evidence that an increase in body metabolism in the female induces increased activity on the part of the ovary and has a marked effect on oestruation (SLONAKER, '24). If it be granted that the ova may be influenced by periodic changes in metabolic activity, it is not necessary to assume that their level of metabolism is altered and that sex is determined by the high or the low level of metabolism in the ova, not by the spermatozoa. — Though the important work of RIDDLE ('16; '17a, b etc.) on pigeons makes this assumption very attractive. Granting that fertilization may be selective, although there is no proof or disproof that such is the case, it seems possible that the admitted periodic changes in body metabolism that occur at different seasons of the year may influence the ova in some way so that they have a greater chemical attraction or repulsion for one kind of spermatozoa than for the other at different times. On this view increased metabolism, which favors reproductive activity, would seem to render the ova more readily fertilized by male-producing than by female-producing spermatozoa. Decreased metabolic activity, which lessens productiveness, would tend to favor the fertilization of the ova by spermatozoa that are female-producing.

So many factors, physiological as well as environmental, act on reproduction that it is difficult, if not impossible, to determine just what alterations in fertility and in the sex ratio can be attributed to temperature and nutrition changes occurring at different periods of the year. Many of the factors that are known to influence reproductive activity were eliminated in the breeding of the various series of Norway rats for which data are given in the present paper, and the series of records is complete enough to show the trend in litter production and in the sex ratio not only during the various months and seasons of the year, but also during the reproductive life of the breeding females. From the find-

ing in these data it appears that there is a pronounced yearly cycle in litter production and in the sex ratio; periods of high productiveness tend to be associated with a high sex ratio more often than with a low one, and vice versa. As a tentative explanation of these facts it is assumed that temperature is the environmental factor that has most influence on reproduction, and that it acts indirectly through its effect on body metabolism. An increase in body metabolism induced by favorable changes in temperature, which may be either towards a higher or a lower level, is assumed to increase reproductive activity and to raise the primary sex ratio through its effect on the ova, not on the spermatozoa. Unfavorable temperature changes, such as extreme and prolonged heat and cold, which lower metabolic activity, correspondingly decrease productivity and also the primary sex ratio on which the sex proportions among the young at birth seem to depend.

Summary.

This paper gives an analysis of series of data for various mammals relative to seasonal variations in fertility and in the sex ratio.

Data given for various races of Norways rats comprise a total of 16 487 litters containing 113 709 individuals. The records cover the entire litter production of a large number of females bred in the animal colony of the Wistar Institute of Anatomy and Biology at various times during the past eighteen years. Data given for other lower mammals, the mouse, pig and dog, were obtained from birth statistics for these animals already published.

The most complete series of data for man is that obtained through the courtesy of Dr. C. B. DAVENPORT, Director of the Eugenics Record Office at Cold Spring Harbor, N. Y. This series comprises unpublished records of 1 983 American families in which there was a total of 11 381 births. A second series of data for man used is that for births in the registration district of the United States during 1915 to 1924 inclusive, as reported by the United States Census. The third series is that for births in Prussia (1872—1881) as given by DÜSING ('84).

In all the various tables the data given are arranged according to the probable time of conception, and the sex ratios are shown as the number of males to each 100 females.

In all of the lower mammals for which date are given fertility, as measured by the number of litters cast, appears to be at its maximum in spring or summer, and at its minimum in autumn or winter. There is, evidently, but one pronounced cycle in fertility during the year.

There does not appear to be any marked correlation between seasonal variations in litter production and litter size. The average size of the litters cast appears fairly constant throughout the year, and does not

seem to be influenced to any extent by the month or season in which conception occurs (chart 2).

In all lower mammals studied, with the exception of the mouse, more litters are conceived, the average size of the litters is greater and the sex ratio is higher during the first half of the year than during the second half (table 6).

The data given for man are conflicting as regards the relative number of conceptions occurring in different months and seasons of the year. The data for the United States registration area and for Prussia (tables 12, 13) indicate a well defined biannual cycle in reproductive activity, with one maximum in summer and the other in the late autumn. The data from the Eugenics Record Office indicates but one yearly 'conception' cycle, with its maximum in November and its minimum in July (table 11).

There is no accord in the series of data for man regarding semi-yearly differences in the number of conceptions and in the sex ratio. The combined data for over 24 000 000 births show a greater number of conceptions in the first half of the year, but a higher sex ratio for conceptions in the second half (table 14).

In the Norway rats, when hybridization does not influence the results, the sex ratio closely approximates the 1 : 1 ratio.

There is a yearly cycle in the sex ratio in all the lower mammals, which is much more pronounced and more important statistically than the seasonal cycle in the sex ratio.

In the various races of Norway rats the yearly cycle in the sex ratio reaches its highest point in conceptions occurring in spring or summer, and falls to its lowest point in autumn or winter conceptions (tables 1 to 4). In the mouse, pig and dog, the sex ratios tend to be high for conceptions in autumn (chart 6). The trend of these ratios is therefore just the reverse of that shown for the rat (chart 5).

There is indication of a direct correlation between fertility and the sex ratio in the data for the Norway rat. Months of low productivity tend to be those in which the sex ratio is also low (table 15); those of high productivity show, as a rule, sex ratios that are above the mean, but not at the highest point. The findings for the mouse, pig and dog are so conflicting that no conclusions can be drawn from them regarding the relation between litter production and the sex ratio.

The findings in the various series of data for man are in apparent hopeless discord. An inverse relation between fertility and the sex ratio is clearly indicated in the data from Prussia, but not in those from the United States Census. The trend of the sex ratio in the Eugenics Record Office series of data accords well with that for the rat (chart 7), but that there is any exact relation between this ratio and the 'conception' curve for the series (chart 3) is not evident.

The seasonal changes in the sexual cycle of lower mammals is attributed to the effect of the rise and fall of temperature on body metabolism, not to nutrition.

The sex ratio of conception (primary sex ratio) in the rat is estimated to be about as high as that in the pig (150 ♂ to 100 ♀).

Prenatal mortality in lower mammals is apparently very great. It seemingly tends to act uniformly throughout the year, since the average size of the litters remains practically the same at all periods of the year. This statement presupposes that in all these mammals about the same number of ova are shed at each ovulation period as is the case in the rat (LONG and EVANS, '22).

Variations in the sex ratio at birth (secondary sex ratio) in the rat are assumed to be due chiefly to variations in the primary sex ratio.

A tentative hypothesis is advanced to account for sesonal variations in the primary sex ratio in the rat. This hypothesis assumes that sex is not determined by a purely chance meeting of the ova and sperm, but that fertilization is selective. Changes in body metabolism induced by favorable or unfavorable temperature conditions are considered to influence the ova and to render them more readily fertilized by one kind of spermatozoa than by the other at different times of the year.

Zusammenfassung.

Die vorliegende Arbeit analysiert eine Reihe von Ergebnissen über jahreszeitliche Schwankungen der Fruchtbarkeit und des Geschlechterverhältnisses bei verschiedenen Säugern.

Die Befunde über verschiedene Rassen der norwegischen Ratte umfassen insgesamt 16 487 Würfe mit 113 709 Individuen. Die Protokolle erstrecken sich auf die gesamte Nachkommenschaft einer großen Anzahl von Weibchen, die in der Tierkolonie des Wistar Instituts für Anatomie und Biologie während der letzten 18 Jahre gezüchtet wurden. Die Angaben über die anderen niederen Säugetiere, Maus, Schwein und Hund, wurden bereits veröffentlichten Geburtsstatistiken für diese Tiere entnommen.

Das vollständigste statistische Material für den Menschen verdanke ich dem Entgegenkommen von Dr. C. B. DAVENPORT, Direktor des Eugenics Record Office in Cold Spring Harbour, N. Y. Dasselbe umfaßt unveröffentlichte Protokolle über 1983 amerikanische Familien, bei welchen eine Summe von 11 381 Geburten aufgezeichnet wurde. Die zweite Reihe gründet sich auf das Geburtenverzeichnis des Registration District der Vereinigten Staaten von 1915 bis einschließlich 1924, wie sie durch den Census der Vereinigten Staaten ausgegeben wird. Die dritte Reihe umfaßt die Angaben über die Geburten in Preußen (1872 bis 1881), die von DÜSING (1884) veröffentlicht wurden.

In all den verschiedenen Tabellen sind die Angaben entsprechend der wahrscheinlichen Zeit der Empfängnis geordnet, und das Verhältnis der Geschlechter wird als Zahl der männlichen Individuen auf je 100 weibliche dargestellt.

Bei allen niederen Säugern, für welche Angaben vorhanden sind, scheint die Fruchtbarkeit, gemessen nach der Zahl der Würfe, ihr Maximum im Frühjahr oder Sommer, ihr Minimum im Herbst oder Winter zu erreichen. Offenbar

gibt es nur einen ausgesprochenen Jahreszyklus der Fruchtbarkeit innerhalb eines Jahres.

Zwischen den jahreszeitlichen Schwankungen in der Zahl und der Größe der Würfe scheint keine ausgesprochene Beziehung vorhanden zu sein. Die Durchschnittsgröße der Würfe erscheint während des ganzen Jahres ziemlich konstant; sie scheint durch den Monat oder die Jahreszeit, in welcher die Befruchtung erfolgte, nicht wesentlich beeinflußt zu werden (Kurve 2).

Alle untersuchten niederen Säuger nehmen, mit Ausnahme der Maus, während der ersten Hälfte des Jahres öfter auf als während der zweiten Hälfte; auch ist im ersteren Fall die Durchschnittsgröße der Würfe größer und das Geschlechterverhältnis höher (Tabelle 6).

Die für den Menschen vorliegenden Angaben sind in bezug auf die relative Zahl der Konzeptionen in verschiedenen Monaten und Jahreszeiten widersprechend. Die Angaben für den Bezirk der United States Registration und für Preußen (Tabelle 12, 13) ergeben einen deutlich ausgesprochenen biannuellen Cyclus der Fortpflanzungstätigkeit mit einem Maximum im Sommer und dem anderen im Spätherbst. Die Angaben aus dem Eugenics Record Office ergeben nur einen jährlichen „Conceptions"cyclus mit dem Maximum im November und dem Minimum im Juli (Tabelle 11).

Hinsichtlich der Angaben über die halbjährlichen Unterschiede in der Zahl der Befruchtungen und in dem Verhältnis der Geschlechter besteht zwischen den drei benutzten Quellen keine Übereinstimmung für den Menschen. Die vereinigten Angaben über mehr als 24 000 000 Geburten zeigten eine größere Anzahl von Befruchtungen in der ersten Hälfte des Jahres, aber ein höheres Geschlechterverhältnis für die Befruchtungen in der zweiten Hälfte des Jahres (Tabelle 14).

Bei der norwegischen Ratte nähert sich das Verhältnis der Geschlechter ziemlich genau dem Wert 1 : 1, wenn die Ergebnisse nicht durch Bastardierung beeinflußt werden.

Bei allen niederen Säugern zeigt das Geschlechterverhältnis einen jährlichen Cyclus, der viel ausgesprochener und auch statistisch viel wichtiger ist als der jahreszeitliche Cyclus des Geschlechterverhältnisses.

Bei den verschiedenen Rassen der norwegischen Ratte erreicht der jährliche Cyclus des Geschlechterverhältnisses seinen Höhepunkt in den Befruchtungen während des Frühjahrs und Sommers und sinkt zu seinem tiefsten Stand in den Befruchtungen im Herbst und Winter (Tabelle 1—4). Bei der Maus, beim Schwein und beim Hund verschiebt sich der Höhepunkt des Geschlechterverhältnisses nach den Konzeptionen im Herbst (Kurve 6). Der Richtungsverlauf der Kurve für diese Verhältnisse ist deshalb gerade umgekehrt wie für die Ratte (Kurve 6).

Eine direkte Beziehung zwischen Fruchtbarkeit und Geschlechterverhältnis ist in den Befunden für die norwegische Ratte angedeutet. Monate mit geringerer Fruchtbarkeit pflegen meist auch ein niedrigeres Geschlechterverhältnis zu ergeben (Tabelle 15): diejenigen mit großer Fruchtbarkeit zeigen für gewöhnlich Geschlechterverhältnisse, die über dem Durchschnitt liegen, aber nicht den höchsten Punkt erreichen. Die Ergebnisse für Maus, Schwein und Hund sind so widersprechend, daß bei ihnen hinsichtlich der Beziehung zwischen Wurfproduktion und Geschlechterverhältnis keine Schlüsse gezogen werden können.

Die Befunde aus den verschiedenen Angabenreihen für den Menschen stehen in scheinbar hoffnungslosem Widerspruch zueinander. Eine umgekehrte Beziehung zwischen Fruchtbarkeit und Geschlechterverhältnis geht deutlich hervor aus den Angaben aus Preußen, aber nicht aus denjenigen des United States Census. Der Kurvenverlauf des Geschlechterverhältnisses in der Angabenreihe des Eugenics Record Office stimmt mit demjenigen für die Ratte gut überein

(Kurve 7); aber es geht daraus nicht hervor, daß eine engere Beziehung zwischen diesem Verhältnis und der „Conceptions"kurve (Kurve 3) vorhanden ist.

Die jahreszeitlichen Veränderungen im Sexualcyclus der niederen Säugetiere werden dem Einfluß der steigenden und fallenden Temperatur auf den Körperstoffwechsel, nicht der Ernährung zugeschrieben.

Das Geschlechterverhältnis bei der Befruchtung (primäres Geschlechterverhältnis) wird für die Ratte ungefähr ebenso hoch geschätzt, als für das Schwein (150 ♂ auf 100 ♀).

Die Mortalität vor der Geburt scheint bei niederen Säugern sehr hoch zu sein. Sie verhält sich scheinbar gleichmäßig während des ganzen Jahres, da die Durchschnittsgröße der Würfe praktisch zu allen Perioden des Jahres die gleiche ist. Dieser Befund legt die Vermutung nahe, daß bei all diesen Säugetieren bei jeder Ovulationsperiode ungefähr die gleiche Anzahl von Eiern ausgestoßen wird, wie das bei der Ratte der Fall ist (LONG und EVANS 1892).

Veränderungen im Geschlechterverhältnis bei der Geburt (sekundäres Geschlechterverhältnis) bei der Ratte werden als hauptsächlich durch Veränderungen des primären Verhältnisses der Geschlechter verursacht aufgefaßt.

Eine Versuchshypothese wird aufgestellt, um die jahreszeitlichen Schwankungen im primären Geschlechterverhältnis bei der Ratte zu erklären. Diese Hypothese nimmt an, daß das Geschlecht nicht bestimmt wird durch ein zufälliges Zusammentretten von Ei und Sperma, sondern daß die Befruchtung selektiv ist. Veränderungen im Körperstoffwechsel, verursacht durch günstige oder ungünstige Temperaturbedingungen, vermögen vermutlich die Eier zu beeinflussen und sie zu verschiedenen Zeiten des Jahres für die eine Art von Spermatozoen leichter befruchtbar zu machen als für die andere.

(Übersetzt von A. HARTMANN-München.)

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